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Potential Antibacterial Use of Selected Seaweed Species Against Aquaculture Pathogens

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ABTRACT

Pathogens in the aquaculture industry have threatened commercially important fish species, leading to significant losses, and conventional drugs used to combat these pathogens are usually expensive. Plants are good medicinal alternatives as they contain bioactive compounds. Seaweeds Ulva spp. and Padina spp. are abundant at the intertidal zones of Nangaramoan Beach in Sta. Ana, Cagayan, and have shown potential inhibitory properties against pathogenic bacteria. Despite their abundance, these remain understudied. This study aimed 1) to evaluate the presence of bioactive compounds in the methanolic and ethanolic extracts from Ulva spp. and Padina spp., and 2) to assess their effectiveness in inhibiting the growth of selected pathogens at various concentrations: 1.0 mg/mL, 0.75 mg/mL, and 0.5 mg/mL. The phytochemical screening of Ulva spp. and Padina spp. utilized qualitative test tube screening methods. The methanolic and ethanolic extracts of both seaweeds were subjected to the paper disc agar diffusion method against two aquaculture pathogens, namely Escherichia coli and Staphylococcus aureus, to assess the antibacterial activities with Amoxicillin (Benedex) and distilled water as the positive and negative controls, respectively. The phytochemical analysis revealed that Ulva spp. methanolic extract contains flavonoids, while its ethanolic extract contains flavonoids and tannins similar to Padina spp. methanolic extract. The ethanolic extract of Padina spp. contains all three bioactive compounds: flavonoids, saponins, and tannins. In addition, the antimicrobial screening revealed that S. aureus is the most susceptible pathogen to both extracts due to factors, such as bacteria type and dosage dependency. While the results showed that inhibitory activity is significantly lower than the positive control ($p>0.05$), the study revealed that Ulva spp. and Padina spp. have promising antibacterial properties. These extracts are, therefore, recommended to be tested against other aquaculture pathogens and further explored for possible drug formulation.

Keywords: *Bioactive Compounds, Phytochemical Analysis, Paper Disc Diffusion Method, Zone Of Inhibition, Antimicrobial Screening*

INTRODUCTION

Aquaculture has experienced rapid growth and is recognized as a primary means of increasing food production in the future (Troell et al., 2013). However, this sector faces challenges caused by various pathogens, leading to bacterial diseases that pose health threats to commercially important fish species and result insignificant economic losses (Pridgeon and Klesius, 2013; Roh et al., 2016). The majority of the drugs employed to combat these pathogens are costly. In addition, pathogens evolve to resist antimicrobials like antibiotics, antivirals, and antifungals, posing a crucial challenge to modern medicine (Yilmaz and Schiffer, 2021). According to Pepi and Focardi (2021), using antibiotics in aquaculture induced antibiotic resistance in the surrounding bacteria in the column water, sediment, and fish-associated bacterial strains. With this, there is a need to explore and develop cheaper and more effective natural antimicrobial agents with better potential, fewer side effects than antibiotics, good bioavailability, and minimal toxicity (Thanigaivel, 2015; Perez et al., 2016).

Numerous studies highlight the potential of plants not only as sources of sustenance but also as sources of chemical compounds found in specific plant parts, which can be utilized as medicines for treating various diseases (Sofowora et al., 2013). According to Saidin et al. (2021), both inorganic and organic antibacterial agents demonstrated identical trends in suppressing both gram-negative and grampositive bacteria; however, their antibacterial effectiveness is also dependent on the species of bacterial strain and respective mechanism owned by each antibacterial agent. In the Philippines, using plant-based traditional medicines remains widespread, attributed to the costeffectiveness of preparing plant-based remedies, its proven efficacy, and the minimal complications associated with its use in treating common ailments.

Seaweeds are macrobenthic marine algae known as prominent components of shallow marine producers (Trono, 1997). These are utilized in several industrial products as raw materials, such as agar, algin, and carrageenan, but are still widely consumed as food in several nations (Vieira et al., 2018). In addition, seaweed showcases antibacterial activities and can function as antioxidants, attributed to the bioactive compounds generated through secondary metabolism. However, the presence of these compounds may vary depending on the season (Adegbaju et al., 2020), environmental factors (Sivagnanam et al., 2015), and extraction solvent (Sobuj et al., 2021).

Green seaweeds, according to Lakshmi et al. (2020), are cheap sources of predominant biomaterials and high-value chemicals, such as polyunsaturated fatty acids, carotenoids, phycobilins, and polysaccharides. For instance, HarshaMohan et al. (2023) stated in their study that Ulva, a green seaweed species, is a good source of vitamins B, proteins, minerals like calcium, potassium, magnesium, sodium, copper, iron, and iodine, as well as dietary fibers and carotenoids. Ulva species are among the most abundant representatives in coastal benthic communities worldwide; however, the genus as a whole has been relatively underexplored (Wichard et al., 2015), with a lack of research on Ulva species, such as U. fasciata and U. reticulata. Aside from the green seaweeds, Ayrapetyan et al. (2021) noted in their study that brown seaweeds are also known to contain bioactive compounds, including sulfated polysaccharides (fucoidans), which are considered promising antimicrobial components for various applications in medicine and the food industry. The study of Rachmawati et al. (2021) found that P. australis contains alkaloids, flavonoids, saponins, tannins, and antioxidants. Still, Padina sp. is one of the understudied brown seaweeds (Hanry et al., 2022).

The abundance of Ulva and Padina along the intertidal zone of Nangaramoan Beach, San Vicente, Sta. Ana Cagayan has been identified in the study of Baleta and Nalleb (2016). This study aimed 1) to evaluate the presence of bioactive compounds in the methanolic and ethanolic extracts from Ulva spp. and Padina spp. collected from August to September, and 2) to assess their effectiveness in inhibiting the growth of selected pathogens at various concentrations: 1.0 mg/mL, 0.75 mg/mL, and 0.5 mg/mL.

MATERIALS AND METHODS

The seaweed samples (Ulva spp. and Padina spp.) were collected from the intertidal zones of Nangaramoan Beach, San Vicente, Sta. Ana, Cagayan, during August to September. The seaweed maceration and methanolic and ethanolic crude extracts extraction were conducted at the Department of Agriculture – Bureau of Fisheries and Aquatic Resources Regional Office 2 - Fisheries Integrated Laboratory Section in Tuguegarao City, Cagayan. The bacteria (Escherichia coli and Staphylococcus aureus) were acquired from Saint Paul University Philippines Science Laboratory, Tuguegarao City, Cagayan, where the antimicrobial screening was also conducted. Finally, the phytochemical analysis was performed at the Department of Science and Technology Regional Standards and Testing Laboratory (DOST-RSTL), Carig Sur, Tuguegarao, Cagayan. The experimental flow process is shown in Figure 1. Samples of Ulva spp. and Padina spp. were collected from the field. These were washed and left to be air-dried for a week and were pulverized for the maceration process. The solutions were filtered and concentrated through the vacuum-pressured rotary evaporator to obtain the crude extracts used for the phytochemical and antimicrobial analysis.

Phytochemical Analysis

About 50 mL of Ulva spp. and Padina spp. methanolic and ethanolic extract were brought to the DOST RO2-RSTL for the phytochemical screening (Guevarra et al., 2005).

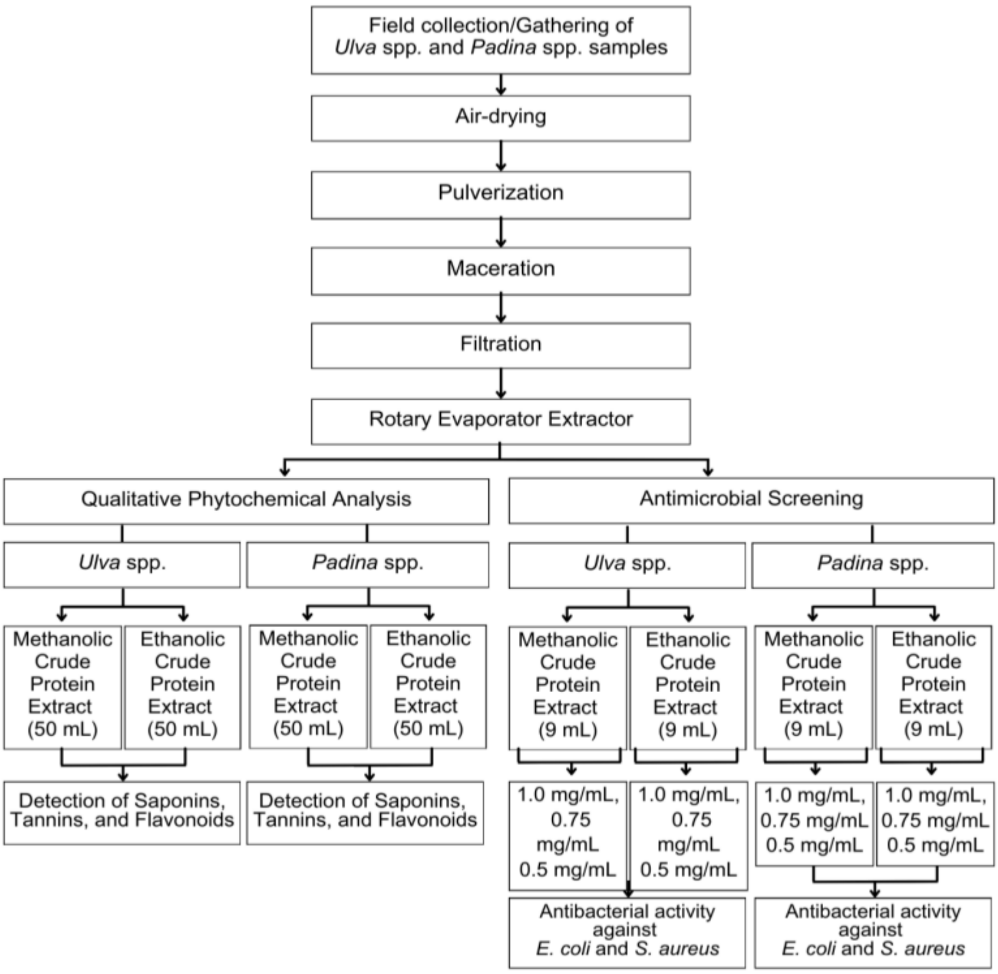

Figure 1. Research Methodological Flow

Preparation of the different concentrations

For the 0.75 mg/L concentration, 2.25 mL of the crude extract was mixed with 0.75 mL of distilled water, while 1.5 mL of the crude extract was mixed with 1.5 mL of distilled water for the .50 mg/L concentration. These dilution procedures were applied individually for each crude extract, resulting in twelve (12) vials.

Antimicrobial Screening

The paper disk agar diffusion method was used to determine the antimicrobial properties of the sample. 3 mL of each ethanolic and methanolic crude extract in different concentrations (assay solution), distilled water (negative control), and amoxicillin (positive control) were dispensed into the sterile paper discs and were performed in five (5) replicates per seaweed species. The impregnated discs were aseptically applied and pressed into the seeded nutrient agar equidistantly. The assay plates were arranged inside the biosafety cabinet. The petri dishes were incubated at 35°C for 20 hours.

After 20 hours of incubation, the diameters of the zones of inhibition (ZOI) were measured in millimeters using a Vernier caliper and with a black paper background. Clear and well-defined ZOI around the discs are observed if the sample tested possessed antibacterial potential, and the failure of the disc to exhibit zones of inhibition indicates the absence of antibacterial effects.

Statistical Analysis

Mean values ± SD were used to present the antibacterial efficacy of the Ulva spp. and Padina spp. extracts against selected aquaculture pathogens based on the average measurement of the ZOI, which were expressed in millimeters using a Vernier caliper. The data were initially tested for normality and homogeneity of variance using Shapiro-Wilk and Kolmogorov-Smirnov tests, respectively. Parametric data were analyzed using Oneway ANOVA followed by Tukey's HSD Test.

RESULTS AND DISCUSSION

The seaweed samples used in the study were identified with the assistance of the DA-Bureau of Fisheries and Aquatic Resources Regional Office 2 based on their morphological characteristics. Two species of Ulva were identified as U. reticulata and U. fasciata, and three species of Padina were identified as P. minor, P. australis, and P. japonica.

Table 1 shows the results of the phytochemical analysis and reveals that Ulva spp. methanolic extract contains flavonoids.

Both Ulva spp. ethanolic extract and Padina spp. methanolic extract contain flavonoids and tannins. The ethanolic extract of Padina spp. contains all three bioactive compounds: flavonoids, tannins, and saponins. The phytochemical analysis was limited to qualitative contents only due to reagent limitations.

Table 1. Result of the Qualitative Phytochemical Analysis

Seaweed Species	Sample Description	Parameter	Result
Ulva spp.	Methanolic crude extract	Flavonoids	+
		Tannins	-
		Saponins	-
	Ethanolic crude extract	Flavonoids	+
		Tannins	+
		Saponins	-
Padina spp.	Methanolic crude extract	Flavonoids	+
		Tannins	+
		Saponins	-
	Ethanolic crude extract	Flavonoids	+
		Tannins	+
		Saponins	+

Table 2 indicates the mean and standard deviation (mean ± SD) of the ZOI (mm) of Ulva spp. extracts in different concentrations (1.0 mg/mL, 0.75 mg/mL, and 0.5 mg/mL) tested on E. coli and S. aureus, wherein the 1.0 mg/mL concentration of both Ulva spp. crude extracts exhibited very active inhibition against S. aureus (methanolic crude extract 20.68 ± 0.975064 and ethanolic crude extract with 25.47 ± 0.92. The 0.75 mg/mL ethanolic crude extract of Ulva spp. exhibited active inhibition with 18.87 ± 1.12, and 0.5 mg/mL exhibited partially active inhibition with 13.24 ± 1.05. On the other hand, all the other crude extracts (Ulva spp. methanolic and ethanolic crude extract in all concentrations against E. coli and methanolic extract at 0.75 mg/mL and 0.50 mg/mL against S. aureus) exhibited inactive inhibition against the selected pathogens.

Table 2. Mean and standard deviation (mean ± SD) of the ZOI of Ulva spp. extracts at different concentrations against selected aquaculture pathogenic bacteria.

Extracts and Concentration		Zone of Inhibition (mm)	
		E. coli	*S. aureus*
Ulva spp. ethanolic crude extract	1.0 mg/mL	6.4 ± 0.42	25.47 ± 0.92
	0.75 mg/mL	6.09 ± 0.12	18.87 ± 1.12
	0.5 mg/mL	6.00 ± 0	13.24 ± 1.05
	Positive Control	18. 79 ± 1.13	28.99 ± 1.92
	Negative Control	---	---
Ulva spp. methanolic crude extract	1.0 mg/mL	6.71 ± 0.5	20.68 ± 0.98
	0.75 mg/mL	6.12 ± 0.27	8.00 ± 1.45
	0.5 mg/mL	6.00 ± 0	7.16 ± 1.10
	Positive Control	19.1 ± 1.42	30.26 ± 1.75
	Negative Control	---	---

Table 3 indicates the mean and standard deviation (mean ± SD) of the ZOI (mm) of Padina spp. extracts in different concentrations (1.0 mg/mL, 0.75 mg/mL, and 0.5 mg/mL) against E. coli and S. aureus. It shows that S. aureus is more susceptible to both the extracts of Padina spp. at 1.0 mg/mL compared to E. coli with 20.65 ± 1.40 (ethanolic crude extract) and 19.80 ± 1.13 (methanolic crude extract), exhibiting very active inhibition. Meanwhile, the 1.0 mg/mL methanolic crude extract against E. coli exhibited active inhibition with 18.41 ± 1.18, and 1.0 mg/mL ethanolic crude extract exhibited partially active inhibition with 10.70 ± 0. 64. The 0.75 mg/mL of the methanolic and ethanolic crude extracts also exhibited active inhibition against S. aureus with 15.05 mm ± 0.21 and 17.42 ± 0.63, respectively. On the other hand, the crude extracts at 0.75 mg/mL concentrations against E. coli exhibited partially active inhibition with 13.98 ± 0.55 (methanolic) and inactive inhibition with 8 ± 0.85 (ethanolic). The 0.5 mg/mL methanolic crude extract exhibited partially active inhibition against E. coli with 10.43 ± 0.55 and against S. aureus with 11.05 ± 0.07. Finally, the 0.5 mg/mL ethanolic crude extract against both pathogens exhibited inactive inhibition.

Table 3. Mean and standard deviation (mean ± SD) of the ZOI of Padina spp. extracts at different concentrations against selected aquaculture pathogenic bacteria.

Extracts and Concentration		Zone of Inhibition (mm)	
		Escherichia coli	*Staphylococcus aureus*
Padina spp. ethanolic crude extract	1.0 mg/mL	10.70 ± 0.64	20.65 ± 1.40
	0.75 mg/mL	8.00 ± 0.85	17.42 ± 0.63
	0.5 mg/mL	6.22 ± 0.38	8.17 ± 0.58
	Positive Control	18.08 ± 1.46	31.73 ± 1.10
	Negative Control	---	---
Padina spp. methanolic crude extract	1.0 mg/mL	18.41 ± 1.18	19.80 ± 1.13
	0.75 mg/mL	13.98 ± 0.55	15.05 ± 0.21
	0.5 mg/mL	10.43 ± 0.55	11.05 ± 0.07
	Positive Control	21.38 ± 0.68	30.55 ± 0.64
	Negative Control	---	---

Phytochemical Analysis

In the present study, Ulva spp. and Padina spp. methanolic and ethanolic crude extracts were subjected to a qualitative analysis for the occurrence of three phytochemicals: flavonoids, saponins, and tannins. The test were only limited to qualitative analysis; no quantification was made due to limitations of available reagents.

The results of the phytochemical analysis revealed that Ulva spp. methanolic extract contains flavonoids. In the studies of Xie et al. (2014) and Górniak et al. (2019), flavonoids have been found to lower adhesion and biofilm formation, porin on the cell membrane, membrane permeability, and pathogenicity, which are considered crucial for the growth of bacteria. The current study also revealed that the ethanolic extracts of Ulva spp. and Padina spp. contain both flavonoids and tannins. As cited in the study of Kaczmarek (2020), tannins are bioactive compounds that interfere with cell metabolism and cause its destruction once these pass through the bacterial cell wall up to the internal membrane. In the current study, the third bioactive compound, saponins, was only present in the ethanolic extract of Padina spp. According to Arabski et al. (2012) and Khan et al. (2018), saponins may enhance the permeability of bacterial membranes, potentially facilitating the influx of antibiotics through the bacterial cell wall membrane. However, the presence of phytoconstituents is mostly determined by numerous factors such as seasonal variations (Adegbaju et al., 2020) and environmental factors (Sivagnanam et al. 2015), as well as the extraction solvents used for brown and green seaweeds (Sobuj et al., 2021). According to Adegbaju et al. (2020), the levels of plant phenolic compounds are affected and significantly altered by seasonal variations in photoperiod, light intensity, and temperature. The collection of the samples was conducted during the rainy months of August to September along the intertidal zones of Nangaramoan beach. Zhang et al. (2020) revealed in their study that total phenolic content is significantly higher during the wet season than during the rest of the year.

Another factor that could affect the presence of extracted bioactive compounds is the solvent used (Sobuj et al., 2021). The current study utilized methanol and ethanol as extraction solvents. According to Prasetyaningrum et al. (2021), solvent ethanol can get the flavonoid content due to its polar side. This supports the current study, which revealed that ethanol was more effective in yielding bioactive compounds than methanol, where ethanol extracted 5 of the 6 bioactive compounds while methanol only extracted 3. In addition, the current study aligns with the study of Nawaz et al. (2020), wherein extraction in highly polar solvents, like methanol, leads to low phenolic (including tannins) and flavonoid content compared to non-polar solvents. Ulva spp. ethanolic crude extract in the present study contains both flavonoids and tannins, whereas its methanolic crude extract only contains flavonoids. The study of Anis et al. (2018) further supports this, revealing that the methanolic extract of U. fasciata contains flavonoids but at a low concentration of 0.0313%.

The type of seaweed used can affect the presence of phytoconstituents. The current study revealed that the methanolic crude extract of Padina spp. exhibited a higher number of bioactive compounds compared to the methanolic extract of Ulva spp. According to Nallamuthu (2013), methanol is particularly suitable for extracting antimicrobials from brown seaweeds. The present study also indicates that the ethanolic crude extract of Padina spp. contains all 3 bioactive compounds. This is supported by the study of Rachmawati et al. (2021) on P. australis, wherein the presence of saponins, tannins, and flavonoids was noted. On the other hand, the methanolic crude extract of Padina spp. in the current study includes flavonoids and tannins but lacks saponins. This finding aligns with the results of Khadijah et al. (2021), where the methanol extract of Padina sp. contains flavonoids and tannins but lacks saponins.

Numerous studies have explored chemical compounds derived from macroalgae, revealing a spectrum of biological activities dependent upon variables such as the specific algae and microorganisms involve (Centeno and Ballantine, 1999; Pooja, 2014). In the study of Remya et al. (2022), brown algae contain bioactive compounds, such as phlorotannin, fucoxanthin, alginic acid, fucoidan, and laminarin, while U. reticulata (green algae) in the study of Julyasih and Purnawati (2023) revealed that terpenoid, phenolic, and alkaloid are the phytochemical compounds present. Moreover, the phytochemical screening in the study of Singkoh et al. (2021) revealed that the ethanolic extracts of brown algae P. australis contained flavonoids, saponins, and tannins and could inhibit the growth of Staphylococcus aureus, S. mutans, and Escherichia coli.

Antibacterial Assay

Algal compounds have been proven to be good sources of bioactive compounds with many potentialities. Numerous studies confirmed that the seaweeds chemical composition and antimicrobial activity vary with geographic location, ecological factors, collection time, and physiological conditions (Pérez et al., 2016). Moreover, Pérez et al. (2016) noted that a lack of antibacterial activity was reported during some seasons. Phaeophyceae showed inactivity in certain seasons, while Chlorophyceae were active throughout the year. In the current study, brown seaweed Padina spp. showed high antibacterial activity against the pathogenic bacteria tested, while the green seaweed Ulva spp. exhibited high antibacterial activity only to S. aureus.

Due to the different content of bioactive compounds as proven in the phytochemical screening, the crude extracts of Padina spp. and Ulva spp. exhibited significantly different inhibitory activity against the fish pathogenic bacteria tested.

Both the ethanolic and methanolic extracts of Padina spp. produced consistent active ZOI against the two pathogenic bacteria, while the extracts of Ulva spp. exhibited active inhibitory activity against S. aureus only, but not against

E. coli. Similar observations were recorded in the study of Srikong et al. (2017), wherein the algal extracts of Padina sp. showed no antibacterial activity against gram-negative bacteria Escherichia coli and Salmonella enteric but showed inhibitory activity against gram-positive bacteria strains (S. aureus), which could be due to the differences in the capability of the extraction protocols to recover any inhibitory phytochemicals and different assay methods, resulting in the different susceptibilities of target bacteria strains.

In the current study, higher inhibition activity yielded by extracts of the brown seaweeds compared to the green seaweeds also suggests that the antibacterial activity depends on the class of seaweed. These findings are similar to the observations of Oumaskour et al. (2012), where several species showing active inhibition against both gram-positive and gram-negative bacteria were recorded in the class of Phaeophyceae rather than Chlorophyceae.

In addition to this, the results of the study also showed the differences in the ZOI of the extracts. The methanolic extract of Ulva spp. showed the lowest inhibition compared to the other extracts was due to the extract containing only one single phytochemical constituent (flavonoids), as compared to the other extracts with two and three bioactive compounds. According to Monteiro et al. (2019), solvent choice is another determinant in the yield of antioxidant activities of algal extracts due to the solvent influence in the solubilization of antioxidant compounds. The results of the current study align with the observation recorded in the study of Nisha et al. (2014), wherein methanol extracts of red and green seaweeds had significantly lower antimicrobial activity than brown seaweed. Pérez et al. (2016) suggested using acetone, while Nisha et al. (2014) suggested using 50:50 ethanol and water, or 50:50 methanol and water for extraction of green seaweed species.

The result of the current study also showed that the inhibitory properties of the seaweed extracts were dose-dependent. Higher concentrations of the different algal extracts exhibited higher inhibitory activities and decreased as the concentration of the seaweed extracts became lower. The same trends in the results were reported in the studies of Vatsos and Rebours (2015) and Gupta et al. (2012). Both the methanolic and ethanolic extracts of Padina spp. and Ulva spp. were found active for all microorganisms tested down to 0.50 mg/mL dilution. However, both extracts of Padina spp. showed higher ZOI for S. aureus compared to E. coli.

The effectiveness of the bioactive compounds is also dependent on the type of organisms. In general, gram-positive bacteria were reported to be more vulnerable to algal extracts than gramnegative bacteria due to the differences in their cell structure and composition (Ali et al., 2020). Gram-positive bacteria were marked by dense peptidoglycan in the outer layer of their cell wall, while gramnegative bacteria have composite, multilayered cell wall structures that make the entry of bioactive compounds more difficult (Arguelles and Sapin, 2020); thus, gram-negative bacteria have higher resistance to antibiotics than gram-positive bacteria (Breijayeh, 2020). In the current study, the extracts showed higher inhibition against the gram-positive bacteria, S. aureus than to the gramnegative bacteria, E. coli; however, the seaweed extracts showed inhibitory activity against both fish pathogenic bacteria tested lower than the positive control.

In the view of the present study, the knowledge of the inhibitory activity of the methanolic and ethanolic crude extracts from Ulva spp. and Padina spp. suggests that these extracts can be utilized as promising sources of antibacterial substances. This also suggests their potential application in aquaculture, particularly in addressing diseases in fish caused by E. coli and S. aureus. Nevertheless, these extracts should be tested against other aquaculture pathogens to comprehensively evaluate their efficacy.

CONCLUSION

On the basis of the findings of the study, the methanolic and ethanolic extracts derived from Padina spp. and Ulva spp. collected along the intertidal zones of Nangaramoan Beach, Sta. Ana, Cagayan, during August to September, possess inhibitory effects against selected aquaculture pathogens: S. aureus and E. coli. These effects are attributed to the phytochemical constituents present, which are known for their antibacterial properties. These bioactive compounds are flavonoids, tannins, and saponins. The current study revealed that Ulva spp. methanolic extract contains flavonoids, and Ulva spp. ethanolic extract contains flavonoids and tannins similar Padina spp. methanolic extract Padina spp. ethanolic extract contains all three bioactive compounds: flavonoids, saponins, and tannins. The study also found that S. aureus demonstrated the highest susceptibility to all extracts compared to E. coli. Specifically, both methanolic and ethanolic crude extracts of Padina spp. and the ethanolic extract of Ulva spp. exhibited partially active inhibition at 0.5 mg/mL against S. aureus. However, the methanolic extract of Ulva spp. showed inactive inhibition at 0.75 mg/mL against S. aureus. The crude extracts of Ulva spp. and the methanolic extract of Padina spp. demonstrated inactive inhibition at 1.0 mg/mL, and the methanolic extract of Padina spp. showed partially active inhibition at

0.75 mg/mL against E. coli. Finally, the study revealed that inhibitory activity is significantly lower than the positive control. Nevertheless, the study implies that the seaweeds Ulva spp. and Padina spp. hold promise for potential exploration and development for both preventive and therapeutic purposes due to their antibacterial attributes, and seaweeds could serve as viable alternatives in developing new drugs.

RECOMMENDATION

The researchers would like to state some recommendations for the institution and other interested researchers. Based on aforementioned findings, the following recommendations are drawn:

1. The use of other extraction methods, solvents, and analysis techniques to identify and quantify the phytochemical constituents, including their antibacterial properties, to provide a more comprehensive understanding of Ulva spp. and Padina spp. chemical compositions.
2. Conduct tests on other seaweeds species, especially those commercially cultured for ease in sample collection, to evaluate their potential inhibitory effects against other aquaculture pathogens (i.e., Aeromonas hydrophila, A. salmonicida, salmonicida, Streptococcus spp., Vibrio spp., etc.).
3. Conduct of additional research on antimicrobial susceptibility methods or confirmatory tests should to identify the antibacterial effectiveness of Ulva spp. and Padina spp. extracts.
4. Further studies should be undertaken using specific Ulva and Padina species taken during the other months. This is to identify which species has the most inhibitory properties or the potential to serve as an antibacterial agent. Additionally, it aims to determine which month exhibits the highest concentration of bioactive compounds.
5. Exploration of bioactive compounds present in seaweeds. The government and the academic community are urged to promote and fund research on bioactive compounds in seaweeds to develop new drugs and address antibiotic resistance.
6. Raise awareness in promoting the sustainable utilization and harvesting of Ulva spp. and Padina spp. The government and relevant departments should launch programs to raise awareness and enhance understanding of the utilization of Ulva spp. and Padina spp., especially in regions where these resources are abundant

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