DOI: 10.31398/tpjf/32.1.2023-0010

SHORT COMMUNICATION

Antibacterial Potential of *Gracilaria edulis* Extracts Against Pathogenic Bacteria: Input to Organic Aquaculture

Nielvin R. Cansejo[1,2] *, Regine G. Calucag[2,3], Emma L. Ballad[1], Glycinea M. de Peralta[2]

[1] *DA-Bureau of Fisheries and Aquatic Resources - RO2, Regional Government Center, Carig Sur, Tuguegarao City, Cagayan, 3500 Philippines*
[2] *Cagayan State University – Aparri Campus, Maura, Aparri, Cagayan, 3515 Philippines*
[3] *DA-Bureau of Fisheries and Aquatic Resources - CAR, Easter Road, Guisad, Baguio City, 2600 Philippines*

ABSTRACT

With pathogenic bacteria in aquaculture becoming increasingly resistant to antibiotics, there is a compelling need to look into bioactive chemicals present in seaweed as novel treatment options for fish infections. This study evaluated the phytochemical characteristics of *Gracilaria edulis* extracts and their antimicrobial activities against selected aquaculture pathogenic bacteria such as *Aeromonas hydrophila, Escherichia coli*, and *Staphylococcus aureus*. The antimicrobial assay test confirmed that both methanolic and ethanolic extracts of *G. edulis* inhibited the bacteria comparable to that of the positive control. The study, therefore, suggests that *G. edulis* can further be investigated for the possible formulation of therapeutics and drugs in light of its potential as an antibacterial agent, particularly for organic aquaculture practices.

*Corresponding Author: *nielvincansejobfar02@gmail.com*
Received: *February 15, 2023*
Accepted: *February 6, 2025*

Keywords: *aquaculture, pathogenic bacteria, ethanolic crude extract, methanolic crude extract, paper disc diffusion method, zone of inhibition*

Several incidences of bacterial pathogens on cultured aquatic species have been reported worldwide. The occurrence of *Staphylococcus aureus* in farmed shrimps required the development of a vaccine to prevent the disease caused by it (Arfatahery et al. 2015). *Escherichia coli* was confirmed in Nile tilapia (*Oreochromis niloticus*) from a fish farm in Bangladesh (Reza et al. 2021). There has also been ample information documented on the impact of *Aeromonas hydrophila* on aquaculture, with significant mortality recorded on Silver carp *(Hypophthalmichthys molitrix)* (Rashid et al. 2013). The predominance of *A. hydrophila*, together with negligible populations of *A. salmonicida* and *A. sobria*, suggests that *Aeromonas* spp. are common commensal bacteria in tilapia and their environment, capable of causing disease epizootics in stressed conditions (Pakingking et al. 2020).

Various chemotherapeutics, vaccines, immunostimulants, and probiotics have been used to treat bacterial diseases in fish farming, but the emergence of mutants and drug-resistant microorganisms has become a major issue. Antibiotic-resistant bacteria are causing an increase in the number of infections worldwide (Levy and Marshall 2004). Decreased efficiency and pathogen resistance to antibiotics have necessitated the development of new alterations (Bolanos et al. 2017).

Studies revealed that seaweeds are rich sources of bioactive compounds, capable of producing secondary metabolites that can be used as antimicrobial agents and have the potential to be used as new pharmaceutical materials (Maftuch et al. 2016). Such bioactive compounds are also referred to as phytochemicals and are primarily responsible for the protection of plants against insect infestations and microbial infections (Escobido et al. 2016). Unlike pharmaceutical chemicals, phytochemicals like alkaloids, anthraquinones, tannins and polyphenols, saponins, flavonoids, steroids, terpenoids, and cyanogenic glycosides have no adverse effects; hence, they are considered “friendly medicines” that play a vital role against numerous diseases (Banu and Cathrine 2015).

Red seaweeds are a good source of bioactive compounds that have potential as antimicrobial agents

(Andriania et al. 2016). Species such as *G. verrucosa* was found to possess alkaloid, flavonoid, tannin, and phenolic compounds and has shown to have moderate antibacterial activity against fish pathogenic bacteria such as *A. hydrophila*, *Pseudomonas aeruginosa*, *P. putida* (Maftuch et al. 2016). *Gracilaria corticata, G. dentata,* and *G. pygmaea* also contain carbohydrates, glycoside, tannin, phenol, saponin, protein, and flavonoid, which have good antimicrobial activity against *E. coli* and *Salmonella typhi* (Qari and Khan 2019).

Gracilaria edulis is widely distributed in coastal areas throughout the Philippines, particularly in the municipalities of Cagayan Province, such as Buguey, Sta. Ana, and Claveria. However, research on *G. edulis* as an antimicrobial substance against aquaculture pathogens and a potential source of bioactive compounds is poorly documented, which is the impetus of this study. This study focused on exploring the bioactive compounds contained in *G. edulis* found and further evaluated its antimicrobial properties against selected aquaculture pathogenic bacteria (*S. aureus*, *E. coli*, and *A. hydrophila)* using methanolic and ethanolic extraction. The results of this research may be utilized as a basis for the formulation of organic drugs with the increasing demand for inputs for organic aquaculture.

The seaweed samples used in the experiment were collected from the Department of Agriculture-Bureau of Fisheries and Aquatic Resources, Claveria Brackishwater Technology Outreach Station (DA-BFAR CBTOS) located in Pata East, Claveria, Cagayan. The collected *G. edulis* samples (~8.85 kg) were washed thoroughly with salt water to remove unnecessary materials like shells, stones, and sand. Cleaned *G. edulis* were allowed to be air-dried for a minimum of a week and cut into small pieces before soaking or maceration. Following the notes of Guevarra et al. (2005), two hundred grams (200 g) of the coarse powdered *G. edulis* were soaked in 400 mL 80% ethanol in an Erlenmeyer flask and another 200 g in 400 mL 80% methanol. The flasks were tightly covered and allowed to stand for 48 hours.

After 48 hours, the macerated coarse powder of *G. edulis* was removed from the solvents and further filtered using a glass funnel using Whatman No. 1 filter paper discs. The filtrate was then concentrated to about 5 ml through a Rotary Evaporator. A flame test was also used to identify if there was still a presence of alcohol in the extracts when it is put into a flame. The ethanolic and methanolic crude extracts were poured into amber vials and stored inside the refrigerator (0-5 °C) until use. The flow process is shown in Figure 1.

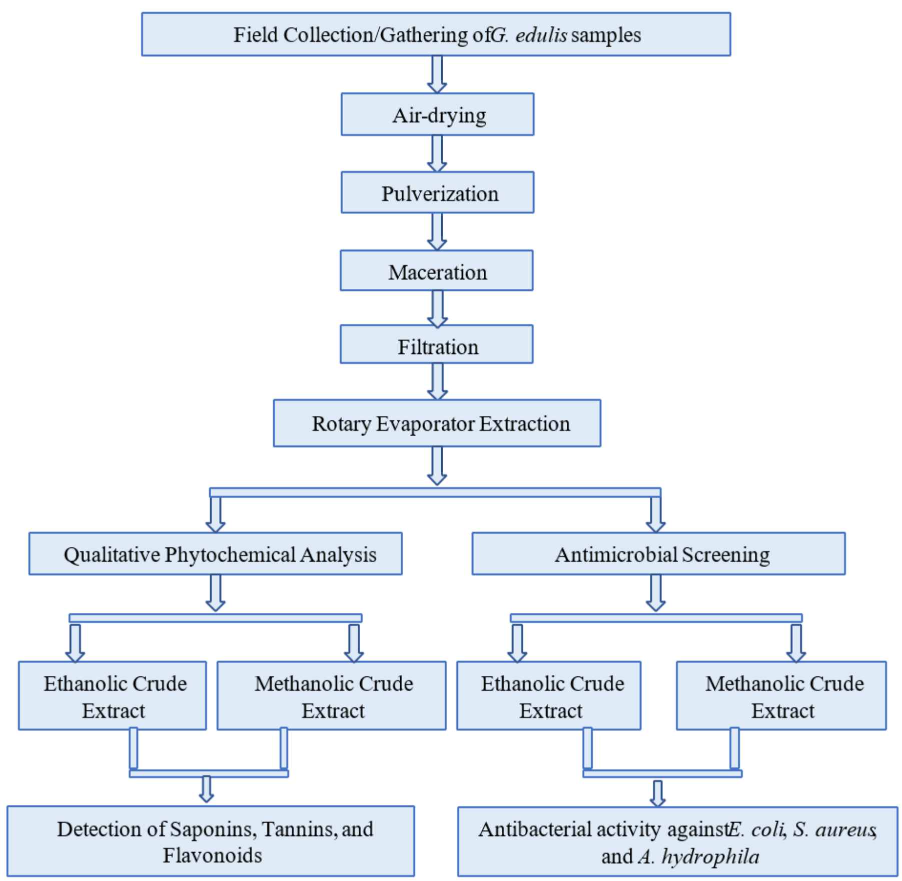

Figure 1. Above is the flow process of the experiment or the research methodological flow from field collection or gathering to air drying of *G. edulis* samples for a week, which have been pulverized for the maceration process. The solution was filtered and concentrated through the rotary evaporator to obtain the crude extracts. Such crude extracts were eventually used for the phytochemical and antimicrobial analysis.

About fifty milliliters (50 mL) of each extract of the *G. edulis* were brought to the Department of Science and Technology Regional Office 02 – Regional Standards and Testing Laboratory (DOST R02-RSTL) for the phytochemical screening following the qualitative screening procedure of Guevarra et al. (2005): ferric chloride test for the detection of tannins; the Bate-Smith and Metcalf method for the test of flavonoids; and the froth test for the detection of saponins.

The paper disc agar diffusion method was performed following the process delineated by Guevarra et al. (2005) to determine the antimicrobial properties of the sample. About 5 mL of ethanolic and methanolic crude extracts (assay solution), distilled water (negative control), and amoxicillin (positive control) were dispensed into the sterile paper discs and were performed in replicates. The impregnated discs were aseptically applied and pressed into the seeded nutrient agar in an equidistant manner. The Petri dishes were incubated at 35 °C. After 24 hours of incubation, the diameters of "zones of inhibition" were measured in millimeters using the Vernier caliper with a black paper background.

Mean values ± SD were used to present the antimicrobial efficacy of the various extracts of *G. edulis* against the selected aquaculture pathogens based on the average measurement of the zones of inhibition, expressed in millimeters using a Vernier caliper. Color intensity was used to indicate the presence of phytochemicals in the methanolic and ethanolic extracts of *G. edulis*. A blue-black color indicates the presence of hydrolyzable tannins, while a brownish-green color may indicate the presence of condensed tannins. A strong red or violet color indicates the presence of flavonoids. However, a "honeycomb" froth greater than 2 cm height from the surface of the liquid that persists after 10 minutes may indicate the presence of saponins.

Table 1 shows the result of the phytochemical analysis of *G. edulis* extracts, which revealed that the ethanolic crude extract of the thallus of *G. edulis* contains tannins only, whereas the methanolic crude extract has flavonoids and tannins. Table 2 indicates the mean and standard deviation of zones of inhibition of *G. edulis* extracts against selected aquaculture pathogenic bacteria: *A. hydrophila*, *E. coli*, and *S. aureus*. Of the three selected aquaculture

Table 1. Result of the qualitative phytochemical analysis.

Sample Description	Parameter	Result	Indicator
Ethanolic Crude Extract	*Flavonoids*	-	Color change (strong red or violet color indicates the presence of Flavonoids; brownish color indicates Tannins)
	Tannins	+	
	Saponins	-	
Methanolic Crude Extract	*Flavonoids*	+	Formation of "honeycomb" froth greater than 2 cm height from the surface of sample that persists after 10 minutes is considered positive for Saponins
	Tannins	+	
	Saponins	-	

Table 2. Mean and standard deviation (Mean ± SD) of zone of inhibition of *G. edulis* extracts against selected aquaculture pathogenic bacteria.

Test Microorganism	Sample Code	Sample Description	Zone of Inhibition (mm)
	MCE	Methanolic Crude Extract	8.26 ± 1.61
A. hydrophila	ECE	Ethanolic Crude Extract	8.00 ± 1.32
	+	Positive Control	7.10 ± 1.27
	-	Negative Control	---
E. coli	MCE	Methanolic Crude Extract	6.70 ± 0.66
	ECE	Ethanolic Crude Extract	6.43 ± 0.61
	+	Positive Control	33.25 ± 0.07
	-	Negative Control	---
S. aureus	MCE	Methanolic Crude Extract	8.10 ± 0.68
	ECE	Ethanolic Crude Extract	6.44 ± 0.70
	+	Positive Control	6.42 ± 0.58
	-	Negative Control	---

Values are means of five replicates (n = 5) ± standard deviation; No inhibition activity (---)

pathogenic bacteria, the *A. hydrophila* was found to be the most susceptible to the ethanolic crude extract as compared to the other bacteria, with a mean ± SD zone of inhibition (ZOI) of 8.00 ± 1.32, followed by *S. aureus* and *E. coli* with means and standard deviations of 6.44 ± 0.70 and 6.43 ± 0.61 respectively. The trend in the responses of these bacteria was also demonstrated to be similar to that of the methanolic crude extract of *G. edulis*, that is, sensitive with *E. coli* as the least susceptible (6.70 ± 0.66), followed by *S. aureus* (8.10 ± 0.68) and *A. hydrophila* (8.26 ± 1.61) as the most susceptible bacterium.

Based on the result, the methanolic crude extract has more phytochemicals than the ethanolic crude extract, which means that the methanol solvent used is more efficient in terms of extracting bioactive compounds from the sample compared to the ethanol. The difference between the two crude extracts in terms of coloration, which determines the presence of phytochemicals for each test, clearly demonstrated this (Figure 2). However, the difference may also be due depending on the quantity of phytochemical compounds found in such extracts (Ballad et al. 2011). Similar results were obtained in the study by Lee et al. (2022), where the methanolic extract showed the highest total polyphenol and flavonoid content compared to ethanol and hot water extracts. The presence of flavonoids in the methanolic crude extract of *G. edulis* in the current observation is also underpinned by the findings of Sobuj et al. (2021), who reported that the methanolic extract of *G. tenuistipitata* contained a significant amount of phenolics and maximum quantity of total flavonoid content (TFC) when compared to ethanol and water extracts, which contained fewer amounts.

According to Do et al. (2013), the phytochemicals may have a higher solubility in methanol than in other solvents. Many phytochemicals, including polar and non-polar compounds, are affected by the polarity of methanol. Based on the principle of similarity and inter-miscibility (like dissolves like), solvents whose polarity values are close to those of the polarity of the solute are likely to perform better, and vice versa (Zhang et al. 2018). It is also important to note that the degree of polarity affects the components of the extracted phytochemicals; otherwise, phytochemicals can be extracted with an appropriate solvent, not to mention that phytochemical flavonoids, alkaloids, and saponins are capable of dissolving in polar solvents such as ethanol and methanol (Dayuti 2018).

Abdalla et al. (2019) also revealed the presence of flavonoids, tannins, cumarin, saponin, and triterpens in the methanolic extracts of *Gracilaria* spp. which the current results validated. These phytochemicals, which include flavonoids, tannins, and saponins, were found in various plants and have been shown to have various biological activities and potential health benefits. According to Shamsudin et al. (2022), flavonoids have been found to exhibit antibacterial properties against various strains of bacteria, including *S. aureus*. It can disrupt bacterial cell walls and membranes, inhibit bacterial enzymes, and chelate iron needed for bacterial growth (Kusmiyati et

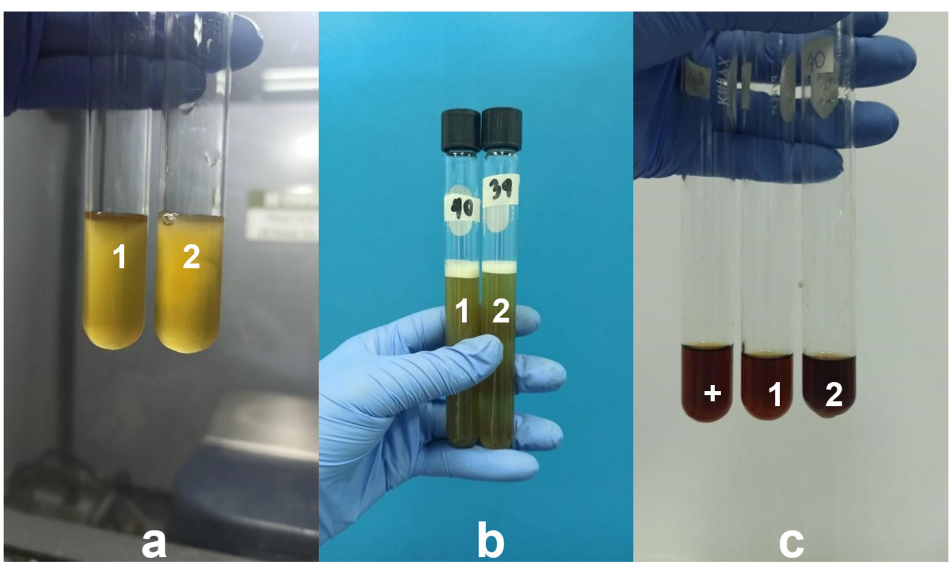

Figure 2. Results of the qualitative phytochemical analysis of sample: (a) ferric chloride for the test of tannins; (b) froth for the test of saponins; (c) Bate-Smith and Metcalf method for the test of flavonoids; (+) control; (1) methanolic extract; (2) ethanolic extract.

al. 2022). Similarly, Syawal et al. (2020) reported that saponins had been discovered to have antimicrobial properties, particularly against fish-pathogenic bacteria such as *A. hydrophila* and *P. aeruginosa*. It can inhibit the growth of bacteria by interacting with their cell membranes, causing disruption and ultimately leading to cell death (Wang et al. 2023). On the other hand, tannins have been reported to have both bacteriostatic and bactericidal effects against harmful bacteria such as *A. hydrophila, E. coli, Listeria, Pseudomonas, Salmonella, Staphylococcus,* and *Streptococcus* (Maisak et al. 2013). This activity is attributed to their ability to complex with polymers and minerals, leading to the inhibition of microbial enzymes and toxicity to bacteria. However, it is significant to note that the minimum inhibitory concentration values of tannins vary depending on the bacterial strain and the source of the tannins.

It is worth noting in the current investigation that the methanolic and ethanolic extracts resulted in higher ZOI for *A. hydrophila* and *S. aureus* when compared to the positive control (Figure 3). This can be due to the phytochemicals present in such extracts, which have the potential to possess antibacterial activity, as reported in the study of Firdausy et al. (n.d.). The difference in inhibition zone, particularly in both extracts, where the ethanolic has shown lesser inhibitory activity when compared with methanolic, is possible due to the presence of a single bioactive compound (tannins), contrary to the latter with two constituents, namely flavonoids and tannins, exacerbated by the lack of saponins with antimicrobial properties (Dayuti 2018; Qari and Khan 2019).

A previous investigation found that Gram-positive bacteria were more affected by seaweed extracts than Gram-negative bacteria (Čagalj et al. 2022). The structure of the cell walls of gram-negative bacteria is more complex, making it difficult for antibacterial compounds to enter the cell and eventually form a firmer inhibition zone (Gonelimali et al. 2018). Though the current investigation showed that *G. edulis* extracts had nearly similar inhibition zones against a Gram-positive bacterium *S. aureus* (Figure 3c) and a Gram-negative bacterium *A. hydrophila* (Figure 3a), it is worth noting that the Gram-negative bacterium *E. coli* (Figure 3b) was the least susceptible.

The present study shows that the efficacy of *G. edulis* extracts against certain pathogenic bacteria shows the alga's potential as a natural treatment in aquaculture. This is especially essential in organic aquaculture, where antibiotic usage is generally regulated or prohibited. The study also suggests that *G. edulis* could be exploited as a natural source of antimicrobial compounds or therapeutics to fight bacterial infections in aquaculture. This could help minimize dependency on antibiotics and slow the spread of antibiotic or antimicrobial resistance.

The present study shows that the efficacy of *G. edulis* extracts against certain pathogenic bacteria shows the alga's potential as a natural treatment in aquaculture. This is especially essential in organic aquaculture, where antibiotic usage is generally regulated or prohibited. The study also suggests that *G. edulis* could be exploited as a natural source of antimicrobial compounds or therapeutics to fight bacterial infections in aquaculture. This could help minimize dependency on antibiotics and slow the spread of antibiotic or antimicrobial resistance.

AUTHORS CONTRIBUTIONS

Cansejo NR: Conceptualization, Methodology, Investigation, Writing - Original Draft. **Calucag RG**: Conceptualization, Methodology, Investigation, Writing - Original Draft. **Ballad EL**: Validation, Writing - Review & Editing, Visualization, Supervision. ***de Peralta GM:*** Validation, Formal Analysis, Supervision.

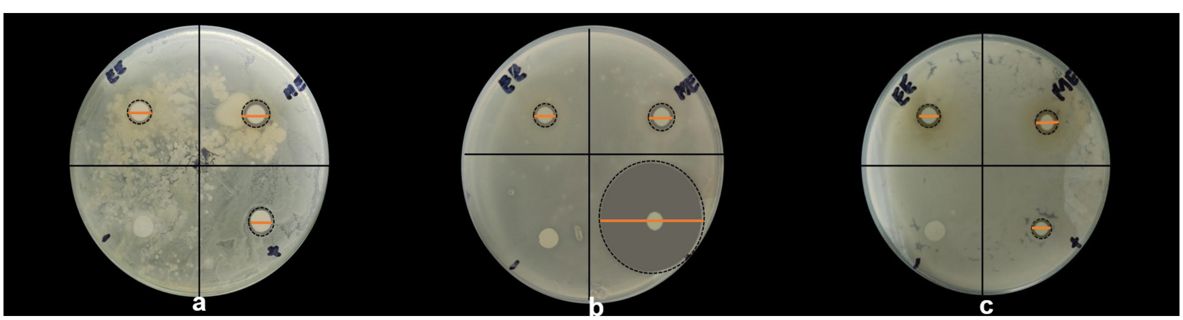

Figure 3. Zone of inhibition of *G. edulis* extracts against selected aquaculture pathogenic bacteria: (a) *A. hydrophila*, (b) *E. coli*, (c) *S. aureus*. Ethanol extract (EE), methanol extract (ME), distilled water as a negative control (-), and amoxicillin as a positive control (+) were used as variables.

CONFLICT OF INTEREST

To the best of our knowledge, no conflict of interest exists.

ETHICS STATEMENT

No animal or human studies were carried out by the authors.

REFERENCES

Abdalla EO, Shigidi MTA. 2019. Phytochemical Screening, Antioxidant Activity and Cytotoxicity of Methanolic Extracts of Selected Red Sea Macroalgae Exhibited Antimicrobial Activities. Haya Saudi J Life Sci. 4(1):39–44. https://doi.org/10.21276/haya.2019.4.1.5

Andriania Y, Syamsumira DF, Yeea TC, Harissonb FS, Herngc GM, Abdullah SA, Oroscoc CA, Alid AM, Latipe J, Kikuzakif H, and others. 2016. Biological Activities of Isolated Compounds from Three Edible Malaysian Red Seaweeds, *Gracilaria changii, G. manilaensis* and *Gracilaria sp*. Natural Product Communications.11(8):1117–1120. https://doi.org/10.1177/1934578X160110082

Arfatahery N, Mirshafiey A, Abedimohtasab TP, Zeinolabedinizamani M. 2015. Study of the Prevalence of *Staphylococcus Aureus* in Marine and Farmed Shrimps in Iran Aiming the Future Development of a Prophylactic Vaccine. Procedia in Vaccinology. 9:44–49. https://doi.org/10.1016/j.provac.2015.05.008

Ballad E, Laureta L, Amar MJ, Toledo N, Pahila I, Aguilar R. 2011. Antibacterial Potential of *Carica papaya*, *Persea americana* and *Azadirachta indica* Extracts against Aquatic Pathogens. Philippine Scientist. 48:35–57. https://www.researchgate.net/profile/Emma-Ballad/publication/350875536_Antibacterial_Potential_of_Carica_papaya_Persea_america na_and_Azadirachta_indica_Extracts_against_Aquatic_Pathogens/links/607ae6a8881fa11 4b40ca040/Antibacterial-Potential-of-Carica-papaya-Persea-americana-and-Azadirachtaindica-Extracts-against-Aquatic-Pathogens.pdf

Banu KS, Cathrine L. 2015. General Techniques Involved in Phytochemical Analysis. International Journal of Advanced Research in Chemical Science. 2(4):25–32. https://www.arcjournals.org/pdfs/ijarcs/v2-i4/5.pdf

Bolanos J, Baleta F, Cairel J. 2017. Antimicrobial Properties of *Sargassum* spp. (Phaeophyceae) against Selected Aquaculture pathogens. International Journal of Current Microbiology and Applied Sciences. 6(2): 1024–1037. http://dx.doi.org/10.20546/ijcmas.2017.602.115

Čagalj M, Skroza D, Razola-Díaz M, Verardo V, Bassi D, Frleta R, Mekinić I, TabanellI G, Šimat V. 2022. Variations in the Composition, Antioxidant and Antimicrobial Activities of *Cystoseira compressa* during Seasonal Growth. Marine Drugs. 20(1):64. https://doi.org/10.3390/md20010064

Dayuti S. 2018. Antibacterial activity of red algae (*Gracilaria verrucosa*) extract against

Escherichia coli and *Salmonella typhimurium*. IOP Conference Series: Earth and Environmental Science. 137(1):012074. https://iopscience.iop.org/article/10.1088/1755-1315/137/1/012074

Do Q, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju Y. 2013. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis. 22:296–302. https://doi.org/10.1016/j.jfda.2013.11.001

Escobido HS, Orbita ML, Orbita R. 2016. Evaluation of the biochemical and phytochemical components of green seaweed *Enteromorpha intestinalis* (Linnaeus) in

Initao, Misamis Oriental, Mindanao, Philippines. International Journal of Biosciences. 9(4):114–122. http://dx.doi.org/10.12692/ijb/9.4.114-122

Firdausy V, Surdano, Pramono H. (n.d.). Activity Test of Crude Extract *Gracilaria* sp. on Propionibacterium acnes Growth Inhibition In Vitro. https://www.academia.edu/41125347/Activity_Test_of_Crude_Extract_Gracilaria_ sp_on_Propionibacterium_acnes_Growth_Inhibition_In_Vitro

Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR. 2018. Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. Front Microbiol. 9:1639. https://doi.org/10.3389/fmicb.2018.01639

Guevarra B, Claustro A, Madulid R, Aguinaldo A, Espeso E, Nonato M, Quinto E, Santos MA, De Castro-Bernas G, Gonzales R, Del Castillo-Solevilla R, Ysrael M. 2005. A Guidebook to Plant Screening: Phytochemical and Biological. pp. 23–98. https://nast.dost.gov.ph/images/pdf%20files/Publications/Outstanding-Awardees%20BOOKS/2006/A%20Guidebook%20to%20Plant%20Screening-%20Phytochemical%20and%20Biological.pdf

Kusmiyati K, Rahmawati E, Waangsir F, Selasa P. 2022. Alkaloids, Flavonoids, Tannins and Saponins Contents in Moringa Oleifera Leaves. Indonesian Journal of Global Health Research. 4(1):139–144. https://doi.org/10.37287/ijghr.v4i1.832

Lee HH, Kim JS, Jeong JH, Park SM, Sathasivam R, Lee SY, Kim CS. 2022. Effect of Different Solvents on the Extraction of Compounds from Different Parts of Undaria pinnatifida (Harvey) Suringar. Journal of Marine Science and Engineering. 10(9):1193. https://doi.org/10.3390/jmse10091193

Levy SB, Marshall B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. Nature Medicine. 10(12):122–129. https://doi.org/10.1038/nm1145

Maftuch M, Kurniawati I, Adam A, Zamzami I. 2016. Antibacterial effect of

Gracilaria verrucosa bioactive on fish pathogenic bacteria. Egyptian Journal of Aquatic Research. 42(4):405–410. https://doi.org/10.1016/j.ejar.2016.10.005

Maisak H, Jantrakajorn S, Lukkana M, Wongtavatchai J. 2013. Antibacterial Activity of Tannin from Sweet Chestnut Wood Against *Aeromonas* and Streptococcal Pathogens of Tilapia (*Oreochromis niloticus*). The Thai Journal of Veterinary Medicine. 43(1):105–111. https://doi.org/10.56808/2985-1130.2440

Pakingking R, Palma P, Usero R. 2020. Aeromonas load and species composition in tilapia *(Oreochromis niloticus*) cultured in earthen ponds in the Philippines. Aquaculture Research. https://doi.org/10.1111/are.14820

Qari R, Khan AR. 2019. Studies on antibacterial and phytochemical analysis of *Gracilaria corticata* (J. Agardh), *Gracilaria dentate* (J.Agardh) and *Gracilaria pygmaea*

(Borgesen) against Diarrheal Causing pathogen *E. coli* and *Salmonella typhi*. Acta Scientific Microbiology. 2(3):3–10. https://www.researchgate.net/publication/331431002

Rashid MM, Hossain MS, Ali MF. 2013. Isolation and identification of *Aeromonas hydrophila* from silver carp and its culture environment from Mymensingh region. Journal of the Bangladesh Agriculture University. 11(2):373–376. http://dx.doi.org/10.3329/jbau.v11i2.19943

Reza R, Shipa SA, Naser N, Miah F. 2021. Surveillance of Escherichia coli in a fish farm of Sylhet, Bangladesh. Bangladesh Journal of Zoology. 48(2):335–346. https://doi.org/10.3329/bjz.v48i2.52373

Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhta RS, Alsharif MA, Parveen H, Zakaria ZA. 2022. Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. Molecules (Basel, Switzerland). 27(4):1149. https://doi.org/10.3390/molecules27041149

Syawal H, Hakim L, Effendi I. 2020. Phytochemical analysis of *Rhizophora apiculata* leaf extract and its inhibitory action against *Staphylococcus aureus, Aeromonas hydrophila* and *Pseudomonas aeruginosa*. AACL Bioflux. 13(4):2242–2249. http://www.bioflux.com.ro/docs/2020.2242-2249.pdf

Sobuj MKA, Islam MA, Islam MS, Islam M, Mahmud Y, Rafiquzzaman SM. 2021. Effect of solvents on bioactive compounds and antioxidant activity of *Padina tetrastromatica* and *Gracilaria tenuistipitata* seaweeds collected from Bangladesh. Sci Rep. 11:19082. https://doi.org/10.1038/s41598-021-98461-3

Wang X, Ma Y, Xu Q, Shikov AN, Pozharitskaya ON, Flisyuk EV, Liu M, Li H, Vargas-Murga L, Duez P. 2023. Flavonoids and saponins: What have we got or missed? Phytomedicine. 109:154580. https://doi.org/10.1016/j.phymed.2022.154580

Zhang QW, Lin LG, Ye WC. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 13:20. https://doi.org/10.1186/s13020-018-0177-x