GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

GSJ: Volume 10, Issue 12, December 2022, Online: ISSN 2320-9186
www.globalscientificjournal.com

POTENTIAL OF Sargassum sp. EXTRACT ORIGIN OF TUNDA ISLAND WATER AS ANTIBACTERIAL OF Vibrio Harveyi WITH IN-VITRO TESTING

Elizabeth Cristina Sitorus[1], Eri Bachtiar[2], Mochamad Rudyansyah Ismail[2], dan M. Untung K. A[2]
[1]Progam studi Ilmu Kelautan, Fakultas Perikanan dan Ilmu Kelautan, Universitas Padjajaran
[2] Departemen Ilmu Kelautan, Fakultas Perikanan dan Ilmu Kelautan Universitas Padjajaran
Jl. Raya Bandung-Sumedang KM 21, Jatinangor, Sumedang Jawa Barat
e-mail : elizabeth.c.sitorus@gmail.com

ABSTRACT

Macroalgae have a broad spectrum of antibacterial activity that can inhibit the Vibrio harveyi bacteria which causes vibriosis disease. Treatment of this disease has been done before using antibiotics or vaccines. However, this treatment has not been effective, so it is necessary to look for other alternatives that can be used as natural antibacterials. The aim of this study was to screen antibacterial using macroalgae extracts of Sargassum sp. from Tunda Island waters against Vibrio Harveyi bacteria in-vitro. The extract material was prepared by using a gradient maceration method using n-hexane, ethyl acetate and acetone solvents, while the secondary metabolite test used the phytochemical method and the in vitro antibacterial test used the Kirby-Bauer method. The results of the antibacterial test that have been carried out show that the three macroalgae extracts of Sargassum sp. has the antibacterial activity of Vibrio harveyi. The highest inhibition zone in the extract of Sargassum sp. is a 75% (7.75 mm) n-hexane extract. The inhibition zone category belongs to the medium category. Phytochemical test results showed that the macroalgae Sargassum sp. contains flavonoids, steroids and saponins. Secondary metabolite compounds are thought to be one of the compounds that act as antibacterial Vibrio harveyi, so testing is necessary to determine the type of specific compounds in the three macroalgae extracts from the waters of Tunda Island.

***Key words**: Antibacterial, Phytochemical, Sargassum, Vibrio harveyi*

PREFACE

Vibriosis disease has affected various species of marine biota which are economically important in various parts of the world during this century. Along with the development of today, knowledge about vibriosis also develops (Mohamad et al., 2019).

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

This disease is caused by vibrio bacteria (Rahmaningsih, 2018). Vibrio bacteria are bacteria belonging to the Vibrinaceae family. These bacteria are rod-shaped and have a flagellum. One of the bacteria belonging to Vibrinaeace is Vibrio harveyi. These bacteria are pathogenic to marine life (Kordi et al., 2010).

To treat vibriosis, farmers usually use antibiotics. One of the antibiotics that is often used is chloramphenicol. Based on previous research chloramphenicol was declared resistant to Vibrio harveyi bacteria (Tendencia & Pena, 2001; Dubert et al. 2015; Wang et al. 2015). Apart from using antibiotics, oral vaccination can also treat vibriosis. However, the use of oral vaccines is less effective because the antigen will dissolve quickly along the fish digestive tract (Sukmawati & Suprapto, 2010).

Therefore, it is necessary to find more effective ingredients to overcome the problems related to vibriosis, which are still occurring today. One of them is by using marine biological materials in the form of macroalgae. According to Manilal et al., 2010 macroalgae have a broad spectrum of antibacterial activity so that they can inhibit Vibrio spp. which is the cause of vibriosis disease.

One type of macroalgae that can be used is the brown macroalgae Sargassum sp. (Mulyadi et al., 2020). Macroalgae Sargassum sp. known to contain active ingredients in the form of secondary metabolites. The secondary metabolites found in one of the sargassum species from Gunug Kidul are flavonoids, tannins, saponins and phenols (Pangestuti et al., 2017). Arsianti et al., 2019 also proved that the macroalgae Sargassum polycystum from Lengkuas Island also contains secondary metabolites consisting of triterpenoids, steroids, alkaloids and tannins. Secondary metabolites contained in Sargassum have bioactivity as anticancer, anti-inflammatory, antioxidant and antibacterial (Sanjeewa et al., 2018). Meanwhile, Sargassum sp. from Trikora Beach, Bintan Island can be used as an antibacterial to inhibit the growth of Vibrio Harveyi bacteria (Naina et al., 2019).

Macroalgae are scattered in various Indonesian waters. One of them is the waters of Tunda Island. Tunda Island is the outermost island bordering Jakarta Bay and Banten Bay which is assumed to get environmental pressure from the development of the area (Dedi et al., 2017). Several things that pose a threat to the ecosystem on Tunda Island include organic and inorganic waste, sedimentation due to sand mining, and exploitation of natural resources. Climate change has also led to the emergence of coral disease, abrasion and sea level rise which can disrupt the balance of environmental carrying capacity (Darus et al., 2014). This will affect the secondary metabolite content and bioactivity of the macroalgae Sargassum sp. origin of the waters of Tunda Island.

Therefore, this research needs to be done to explore and study the secondary metabolites contained in Sargassum sp. origin of the waters of Pulau Tunda, so that you can know the nature of its bioactivity as an antibacterial against one of the bacteria that causes vibriosis, namely Vibrio harveyi.

METHOD

Time and Place of Implementation

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

This research was conducted from May 2019 to January 2020 at the Laboratory of Bioprocess and Bioprospection of Natural Materials, Building 4, 3rd Floor, Faculty of Fisheries and Marine Sciences, Padjajaran University and Padjajaran University Central Laboratory. Macroalgae sampling was carried out on Tunda Island, Banten (Figure 1)

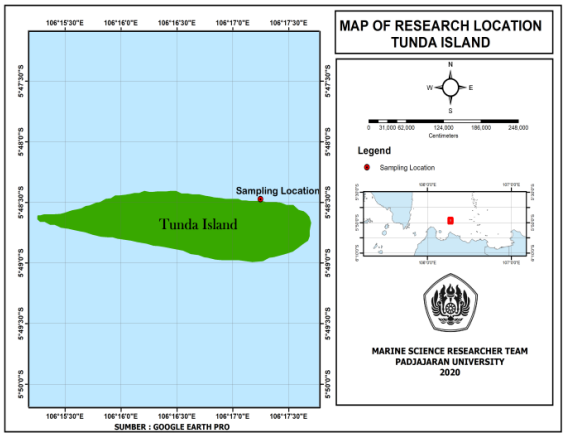

Figure 1. Map of research location

Tools and materials

The tools used in this research are GPS, Rotary evaporator, glass funnel, vial bottle, dropper, measuring cup, test tube, tube rack, tube clamp, bunsen, dropper pipette, drop plate, filter paper, analytical balance, label paper, micropipette, micropipette tip, petri dish, ose needle, paperdisk, incubator, caliper, gloves, mask, erlenmeyer and aluminum foil.

The materials used in this study were samples of Sargassum sp. origin of Tunda Island waters, N-hexane, ethyl acetate, acetone, meyer reagent, Lieberman burchard reagent, 10% ammonia, 1N HCl, CHCl3, 20% HCl, FeCl3, Mg powder, Aquades, DMSO, Vibrio harveyi bacteria, Nutrient Broth, Nutrient Agar and chloramphenicol.

Extraction Procedure of Sargassum sp.

The extraction procedure is carried out by the maceration method in a gradient according to the polarity level. In this research, starting from the solvent n-hexane then ethyl acetate and finally acetone. Each powder weighing 150 grams was soaked for 1x24 hours with a ratio of powder to solvent 1: 5 then filtered and repeated three times. The ratio of 1: 5 was chosen because according to the method used by Rifai et al., 2018 the treatment of the ratio of materials to 1: 5 solvent can produce the highest yield compared to the ratio of 1:10 in 1:15. Then the filtrate obtained from each solvent is concentrated using a rotary evaporator with a temperature of 50˚C and a speed of 120 rpm to form a concentrated extract (Sami, Soekamto, Firdaus, & Latip, 2019 with modifications). After that the yield value is calculated using a formula that refers to Naina et al., 2019 using units (%) where (weight of concentrated extract: initial weight of sample) x 100%.

Phytochemical Test Procedure

To determine the presence of secondary metabolites in Sargassum sp., phytochemical methods were used. Secondary metabolites tested include alkaloids,

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

flavonoids, phenols, triterpenoids & steroids, saponins and tannins. The results obtained are in the form of color changes and can be compared with positive indicators (Agustina, Nurhamidah, & Handayani, 2017).

Antibacterial Test Procedure

The antibacterial test procedure was carried out using the Kirby-bauer agar diffusion method with two repetitions. The procedure used was a 100 µL liquid culture of the test bacteria with a density of 107 CFU / ml which was inserted into NA agar. Then a paper disk was prepared and given each extract of n-hexane, ethyl acetate and acetone from Sargassum sp., Padina sp., And Halimeda sp. which was dissolved using DMSO. Each of these extracts had previously been prepared in concentrations of 100% (50 mg / 50 µl), 75% (37.5 mg / 50 µl), 50% (25 mg / 50 µl) and 25% (12.5 mg / 50 µl). The use of this concentration was chosen because during the preliminary test the concentration of 0.1% (0.05 mg / 50 µl) did not produce an inhibition zone. Furthermore, the paper disk that has been given the extract is placed into a petri dish and stored in an incubator in an inverted state for 24 hours at 37 ° C. The positive control used was chloramphenicol and the negative control used was DMSO (Baleta, Bolaños, Ruma, Baleta, & Cairel, 2017 with modification).

RESULTS AND DISCUSSION

Rendement

The highest extraction yield in the Sargassum sp. Sample was found in ethyl acetate extract with a percentage of 0.33% and the lowest yield in acetone extract with a percentage of 0.13%. Different things were found in previous studies. Macroalgae extract of Sargassum sp. obtained from UD. Seaweed Mandiri, Gunungkidul yields a yield of 0.86% for polar solvents (methanol), 0.77% for semipolar solvents (ethyl acetate) and 0.66% for nonpolar solvents (n-hexane) (Pangestuti, Sumardianto, & Amalia , 2017). This shows that the active compounds contained in the macroalgae Sargassum sp. the origin of the waters of Tunda Island is more semi-polar which is indicated by the high yield in the semi-polar fraction, namely ethyl acetate.

Tabel 1 Rendement of extract *Sargassum* sp.

Sample	Solvent	Rendement	Extract Color
Piece of Sargassum sp.	n-hexane	0,15 %	Golden brown
Piece of Sargassum sp.	Ethyl acetate	0,33 %	Deep black
Piece of Sargassum sp.	Acetone	0,13 %	Deep black

Secondary Metabolite Content of Sargassum sp.

Secondary metabolite content in macroalgae Sargassum sp. identified through phytochemical tests. Phytochemical tests include alkaloids, flavonoids, triterpenoids & steroids, phenols, tannins and saponins. The results of the phytochemical test in this study

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

are shown in table 2. Based on phytochemical testing, macroalgae Sargassum sp. origin of the waters of Pulau Tunda positive for secondary metabolites of flavonoids, steroids and saponins.

Table 2. Phytochemical test results

Test	Result	Description
Alkaloids	-	No sediment
Flavonoids	+	yellowness
Terpenoids & Steroids	+ (Steroid)	Greenish blue color
Saponins	+	Formed foam
Tannins	-	Brown color
Phenol	-	Orange color

Antibacterial Bioactivity Test

Based on the antibacterial activity test of the three extracts of Sargassum sp. originating from the waters of Tunda Island which includes n-hexane extract, ethyl acetate extract and acetone extract against Vibrio harveyi bacteria, it can be seen that the three extracts can inhibit the growth of Vibrio harveyi bacteria (table 3).

Based on table 3, this study shows that n-hexane extract has higher antibacterial activity compared to acetone and ethyl acetate extracts. This indicates that the n-hexane solvent is a suitable solvent for antibacterial activity against Vibrio harveyi bacteria. One of the factors that influence the antibacterial activity is the concentration factor. Zuhud et al., 2001 suggested that high concentrations will produce a high inhibition zone diameter because this will accelerate the release of antibacterial compounds. However, in this study, the concentration of 100% resulted in a low inhibition zone diameter. this is because the extract does not dissolve homogeneously so that the secondary metabolites are not completely absorbed in the paper disk. This is in accordance with Herwandi et al., 2019 which stated that the diffusion power of the extract is one of the factors that affect the inhibition power.

The same thing has also happened in the research of Rahman et al., 2012. According to their research, extracts with high concentrations but not producing a high inhibition zone are caused because the extract is too concentrated and not homogeneous

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

so that even though the concentration increases, the active ingredients can diffuse into the medium is less and finally the formation of the inhibition zone is also a little.

DMSO negative control proved to have no antibacterial activity, so it was confirmed that the antibacterial activity produced was purely from the extracts of the acetone, ethyl acetate and n-hexane fractions of Sargassum sp.

In this study, 24-hour measurements were carried out and 48-hour measurements. After 48 hours of measurement, the bacterial inhibition zone did not decrease. Based on Zaidi et al., 2019 the bacterial inhibition zone that remains at 48 hours shows antibacterial properties in the form of bactericidal. This shows that the extract of Sargassum sp. the origin of the waters of Tunda Island is bactericidal.

According to Pelczar and Chan, 2005 general antibacterial activity causes damage to cell walls, changes cell permeability, changes protein and nucleic acid molecules, inhibits enzyme work and inhibits the synthesis of nucleic acids and proteins.

Inhibition zone produced by macroalgae Sargassum sp. the origin of the waters of Tunda Island is thought to be due to secondary metabolite activity. Based on the results of the phytochemical test, macroalgae Sargassum sp. the origin of the waters of Pulau Tunda contains secondary metabolites of flavonoids, steroids and saponins. The three types of secondary metabolites have different bacterial inhibitory mechanisms.

Table 3. The results of the antibacterial test of Sargassum sp. against Vibrio harveyi

Ekstrak	Konsentrasi	Diameter Zona Hambat (mm)		Diameter rata-rata	Standar deviasi
		Pengulangan ke-1	Pengulangan ke-2		
n-Heksan	100%	1,4	1,4	1,4	0
	75%	7	8,5	7,75	0,75
	50%	8	2	5	3
	25%	3	3	3	0
Etil Asetat	100%	2,7	3,5	3,1	0,4
	75%	6	3	4,5	1,5
	50%	3	2	2	0,5
	25%	1	1	1	0
Aseton	100%	0,1	1,1	0,6	0,5
	75%	3	3	3	0
	50%	1	5	3	2
	25%	1	1	1	0
Kloramfenikol	500 ppm	14,5	14,4	14,4	0,05

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

DMSO	10%	-	-	-

The inhibition mechanism of flavonoid secondary metabolites against Vibrio harveyi bacteria has been previously stated by Vikram et al., 2010. According to these researchers, flavonoid compounds are able to change various physiological processes of bacteria to inhibit bacterial growth, including by inhibiting the formation of biofilms in Vibrio harveyi bacteria and can inhibit them. the formation of a Thype Three Secretion System (TTSS) which is commonly used by these bacteria for self-protection.

Flavonoids can inhibit bacteria by activating microbial adhesin, enzymes and protein transport to microbes. This can occur when flavonoids form complex compounds with extracellular proteins through hydrogen bonds or the formation of covalent bonds (Kumar & Pandey, 2013). In addition, flavonoids can also cause damage to the permeability of bacterial cell walls, lysosomes and microsomes which are the result of the interaction between flavonoid secondary metabolites and bacterial DNA (Sabir 2005).

The mechanism of action of steroids in inhibiting microbes is by damaging the plasma membrane of microbial cells, thereby causing the cytoplasm to leave the cell which in turn causes cell death. It is suspected that this is because steroid molecules have nonpolar (hydrophobic) and polar (hydrophilic) groups so that they have a surfactant effect that can dissolve the phospholipid components of the plasma membrane. It should also be noted that phospholipids are the most dominant constituent components of microbial cell plasma membranes (Wiyanto 2010).

The mechanism of saponins as antibacterials is that they can cause protein and enzyme leakage from within cells. Saponins also have surface active substances that are similar to detergents, so that saponins will reduce the surface tension of the bacterial cell walls and damage the permeability of the bacterial membrane. Saponins will diffuse through the outer membrane and cell wall, then will bind to the cytoplasmic membrane so that it disrupts the cell membrane stability system. This is what causes the cytoplasm to leak out of the cell, causing cell death. The mechanism carried out by these saponins has bactericidal properties (Ningsih et al., 2016). This was also stated by Allam et al., 2017 which stated that the antibacterial activity of saponins could be known based on the surface active properties of saponins. Saponins can enter into the lipid bilayer and bind to cholesterol to form a domain that is rich in saponin cholesterol complexes and finally can carry out cell lysis process.

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

CONCLUSION

Based on the research results, the content of secondary metabolites found in macroalgae Sargassum sp. are flavonoids, steroids and saponins. The highest inhibition zone of Sargassum sp. The origin of the waters of Tunda Island is in the n-hexane fraction at a concentration of 75% with an inhibition zone diameter of 7.75 mm.

BIBLIOGRAPHY

Agustina, W., Nurhamidah, & Handayani, D. (2017). Skrining Fitokimia dan Aktivitas Antioksidan Beberapa Fraksi dari Kulit Batang Jarak (Ricinus communis L.). *ALOTROP Jurnal Pendidikan dan Ilmu Kimia*, *1*(2), 117–122.

Allam, N. G., Abou-taleb, H. K., & Aboobia, M. M. (2017). Antimicrobial and pesticidal activities of soya saponin. *Journal of Basic and Environmental Sciences*, *4*, 262–267.

Arsianti, A., Fadilah, F., Bahtiar, A., Dewi, M. K., Adyasa, Z. M., Simadibrata, D. M., & Amartya, D. (2019). Phytochemistry profile and in vitro cytotoxicity of seaweed macroalgae Sargassum polycystum against colon HCT-116 and lung A-549 cancer cells. *International Journal of Green Pharmacy*, *13*(2), 141–146.

Baleta, F. N., Bolaños, J. M., Ruma, O. C., Baleta, A. N., & Cairel, J. D. (2017). Phytochemicals screening and antimicrobial properties of Sargassum oligocystum and Sargassum crassifolium Extracts. *Journal of Medicinal Plants Studies*, *5*(1), 382–387

Darus, R. F., Dedi, Juraij, Syahrial, Lestari, D. F., Nugraha, A. H., & Zamani, N. P. (2014). Keanekaragaman Hayati Ekosistem Pesisir di pulau tunda, Kabupaten Serang, Banten. *Prosiding Pengelolaan Sumber Daya Kelautan dan Perikanan Wilayah Pesisir dan Pulau-Pulau Kecil Berkelanjutan Menuju Kedaulatan Maritim*, (August), 1–10.

Dedi, Zamani, N. P., & Arifin, T. (2017). hubungan parameter lingkungan terhadap gangguan kesehatan karang di pulau tunda – banten. *Jurnal Kelautan Nasional*, *11*(2), 105–118.

Dubert, J., Osorio, C. R., Prado, S., & Barja, J. L. (2015). Persistence of Antibiotic Resistant Vibrio spp. in Shellfish Hatchery Environment. *Environmental Microbiology*, *4*, 851–860.

Herwandi, Mahyarudin, & Effiana. (2019). Uji aktivitas antibakteri ekstrak etanol annona muricata linn. terhadap vibrio cholerae secara in vitro. *Majalah Kedokteran Andalas*, *42*(1), 11–21.

Kordi, M. Ghufran H., dan Andi Tamsil. 2010. Pembenihan Ikan Laut Ekonomis Secara Buatan. Yogyakarta : ANDI

Kumar, S., & Pandey, A. K. (2013). Chemistry and Biological Activities of Flavonoids : An Overview. *The ScientificWorld Journal*, *2013*, 1–16.

Manilal, A., Sujith, S., Selvin, J., Kiran, G. S., Shakir, C., & Lipton, A. P. (2010). Antimicrobial potential of marine organisms collected from the southwest coast of India against multiresistant human and shrimp pathogens. *Scientia Marina*, *74*(June), 287–296.

Mohamad, N., Noor, M., Amal, A., & Yasin, I. S. (2019). Vibriosis in cultured marine fishes : a review. *Aquaculture*, *512*(May).

Mulyadi, Nur, I., & Iba, W. (2020). Efficacy of Seaweed (Sargassum sp.) Extract to

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

Prevent Vibriosis in White Shrimp (Litopenaeus vannamei) Juvenile. *International Journal of Zoological Research*, *1*, 1–11.

Naina, Y., Wulandari, R., & Said, T. (2019). Skrining Komponen Bioaktif Ethanol 96% Sargassum sp. sebagai Antibakteri Terhadap Vibrio Harveyi. *Intek Akuakultur*, *3*(2), 22–33.

Ningsih, D. R., Zusfahair, & Kartika, D. (2016). Identifikasi Senyawa Metabolit Sekunder serta Uji Aktivitas Ekstrak Daun Sirsak sebagai Antibakteri. *Molekul*, *11*(1), 101–111.

Pangestuti, I. E., Sumardianto, & Amalia, U. (2017). Skrining Senyawa Fitokimia Rumput Laut Sargassum sp. dan Aktivitasnya Sebagai Antibakteri Terhadap Staphylococcus aureus dan Eschericia coli. *Indonesian Journal of Fisheries Science and Technology (IJFST)*, *12*(2), 98–102.

Rahman, D. T., Sutrisna, E. M., & Candrasari, A. (2012). Uji Efek Antibakteri Ekstrak Etil Asetat dan Kloroform Meniran (Phyllanthus niruri Linn) Terhadap Pertumbuhan Bakteri Staphylococcus aureus ATCC 6538 dan Escherichia coli ATCC 11229 Secara in vitro. *biomedika*, *4*(2), 18–25.

Ramhaningsih, Sri. 2018. Hama & Penyakit Ikan. Yogyakarta : Deepublish

Rifai, G., Wayan, I. R. W., & Ayu, K. N. (2018). Pengaruh jenis pelarut dan rasio bahan dengan pelarut terhadap kandungan senyawa fenolik dan aktivitas antioksidan ekstrak biji alpukat (Persea americana Mill.). *Fakultas Teknologi Pertanian, Universitas Udayana*

Sabir, A. (2005). Aktivitas antibakteri flavonoid propolis Trigona sp terhadap bakteri Streptococcus mutans (in vitro). *Maj. Ked. Gigi. (Dent. J.)*, *38*(3), 135–141.

Sami, F. J., Soekamto, N. H., Firdaus, & Latip, J. (2019). Uji Aktivitas Antioksidan Ekstrak Alga Coklat Sargassum polycystum dan Turbinaria deccurens Asal Pulau Dutungan Sulawesi Selatan Terhadap Radikal DPPH. *Jurnal Kimia Riset*, *4*(1), 1–6.

Sanjeewa, K. K. A., Kang, N., Ahn, G., Jee, Y., Kim, Y., & Jeon, Y. (2018). Food Hydrocolloids Bioactive potentials of sulfated polysaccharides isolated from brown seaweed Sargassum spp in related to human health applications : A review. *Food hydrocolloids*, *81*, 200–208.

Sayuti, M. (2017). Pengaruh Perbedaan Metode Ekstraksi , Bagian Dan Jenis Pelarut Terhadap Rendemen Dan Aktifitas Antioksidan Bambu Laut (Isis Hippuris). *Technology Science and Engineering Journal*, *1*(3), 166–174.

Sukmawati, T. D., & Suprapto, H. (2010). Efektivitas Penggunaan Whole Cell dari Vibrio alginolyticus Sebagai Vaksin Oral melalui Artemia pada Benih Ikan Kerapu Tikus (Chromileptes altivelis). *Jurnal Ilmiah Perikanan dan Kelautan*, *2*(2), 113–116.

Tendencia, E. A., & Pena, L. D. De. (2001). Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture*, *195*, 193–204.

Vikram, A., Jayaprakasha, G. K., Jesudhasan, P. R., Pillai, S. D., & Patil, B. S. (2010). Suppression of bacterial cell – cell signalling , biofilm formation and type III secretion system by citrus flavonoids. *Journal of Applied Microbiology*, *109*, 515–527.

Wang, R. X., Wang, J. Y., Sun, Y. C., Yang, B. L., & Wang, A. L. (2015). Antibiotic resistance monitoring in Vibrio spp . isolated from rearing environment and intestines of abalone Haliotis diversicolor. *MPB*, *101*(2), 701–706.

Wiyanto, D. B. (2010). Uji Aktivitas Antibakteri Ekstrak Rumput Laut Kappaphycus alvarezii dan Eucheuma denticullatum Terhadap Bakteri Aeromonas hydrophila

GSJ: Volume 10, Issue 12, December 2022
ISSN 2320-9186

dan Vibrio harveyii. *Jurnal Kelautan*, *3*(1), 1–17.

Zaidi, S. T. R., Weier, N. E., Yorkshire, W., Kingdom, U., & Pharmacy, D. (2019). Bacterial Infections and the Role of the Pharmacist. In *Elsevier* (hal. 730–741).

Zuhud, E. A. ., Rahayu, W. P., Wijaya, C. H., & Sari, P. P. (2001). Aktivitas Antimikroba Ekstrak Kedawung (Parkia roxburghii G. Don) terhadap Bakteri Patogen. *Jurnal Teknologi dan Industri Pangan*, *12*(1), 6–12.