Antibacterial activity of *Eucheuma spinosum* extract against *Vibrio alginolyticus* and *Aeromonas hydrophila*

Irwan Effendi, Muhammad R. Prayogi, Aras Mulyadi

Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia.
Corresponding author: M. R. Prayogi, muhridhoprayogi27@gmail.com

Abstract. *Eucheuma spinosum* is a red seaweed that has economic value because of its very wide use as a food ingredient, in the organic fertilizer industry, cosmetics, textiles, and medicine. *Vibrio alginolyticus* and *Aeromonas hydrophila* are aquatic pathogenic bacteria that can cause various infectious diseases in humans and fishes. This study aimed to determine the group of bioactive compounds contained in spinous branches and their antibacterial activity against these two pathogens. This research was carried out in March-May 2021 and used a completely randomized design (CRD) with 2 factors, namely *E. spinosum* extract concentration and the species of the pathogens. The first factor consists of 6 levels, namely 12.5% (A1), 25% (A2), 50% (A3), and 100% (A4) of *E. spinosum* extract, negative experimental control (NEC; water without any extract), and positive experimental control (PEC; water containing chloramphenicol 10%). The second factor consisted of *V. alginolyticus* (B1) and *A. hydrophila* (B2) with 3 times replications. The results of the extraction of the seaweed with ethanol only obtained two groups of secondary metabolites, namely the group of alkaloids and flavonoids. These two substances acted as an antibacterial against the growth of the bacteria. The highest inhibition zone was for *V. alginolyticus* at a concentration of 50% (A3B1) and the lowest one was at a concentration of 12.5% (A1B1). While for *A. hydrophila* the highest and the lowest inhibition zone were at a concentration of 50% (A3B2) and 100% (A4B2), respectively.
Key Words: antibacterial assay, medicinal herbs, pathogenic bacteria, red algae, secondary metabolites.

Introduction. Seaweed has been widely used as a food ingredient, raw material for making drinks, and ingredients for the pharmaceutical industry. *Eucheuma spinosum* is used as a raw material for making agar, carrageenan, and alginate flour. Agar, carrageenan, and algin (alginate) are widely used in the textile and cosmetic industries. These algae are also economically important because they contain polysaccharide compounds, such as carrageenan, alginate, agar, and agarose types (Hijaz 2009; Ndobe et al 2020). Its main function is as a stabilizing agent, emulsifying agent, thickening agent, filler, and gelling agent. Polysaccharides from several marine algae are also known to have biological activities related to their pharmacological potential as anticoagulants, antioxidants, and antitumors (De Souza et al 2007). In the food industry, these three products (agar, carrageenan, and alginate) are widely used for the manufacture of bread, soup, sauce, ice cream, jelly, candy, cheese, pudding, jam, beer, wine, coffee, and chocolate (Liao et al 2021).

E. spinosum is a seaweed that has economic value because of its wide use, as a food ingredient, in the organic fertilizer industry, cosmetics, textiles, and medicine. The use of seaweed is caused by the presence of carrageenan which acts as a stabilizer, thickener, chelating agent, emulsifier, and others. This alga belongs to the red algae group that has primary and secondary metabolites of high economic value, such as hydrocolloid compounds used in the food, pharmaceutical, and cosmetic industries. It can be used as raw material for the manufacture of carrageenan polysaccharides. Such properties of carrageenan cause this material to be widely used by industry to obtain higher-quality products (De Souza et al 2007; Muawanah et al 2016; Wijayanto et al 2020).

Manuel et al (2014) used seaweed (*E. cottonii* and *E. spinosum*) to increase the iodine and dietary fiber content of fish meatballs to help fulfill the daily needs of Indonesian consumers. Damongilala et al (2021) and Inayah & Masruri (2021) reported that the *E. spinosum* extracts had antioxidant compounds based on free radical scavenging activities using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and superoxide dismutase (SOD). This extract was indicated to contain powerful antioxidants since the ethyl acetate extract scavenged DPPH and SOD free radicals. A pure compound, 3-(3-methoxyphenyl) propanal, was isolated, having antioxidant properties. *E. spinosum* contained natural antioxidants which have the potential to be developed as a functional food and disease prevention and treatment. The crude extract of polysaccharides of *E. cottonii* and *E. spinosum* has the potential to be developed as an alternative antioxidant and anticancer agent (Muawanah et al 2016).

Vibrio alginolyticus is one of the most pathogenic species of Gram-negative halophilic bacteria. It is found mainly in estuaries, and coastal and aquatic environments (Narracci et al 2013). The cell populations are very large, some are free-living, and others are parasitic or associated with the body surface of organisms such as vertebrates, marine invertebrates and flora, and even humans (Chen et al 2011; Schets et al 2011). It causes various infections and inflammations in humans and animals (Wei & Wendy 2012), like in *Penaeus vannamei* (Pereira et al 2007).

Aeromonas hydrophila is one of the most important bacterial pathogens in freshwater fishes in Indonesia and perhaps the most common organism associated with the aquatic environment. It is a non-spore-forming, Gram-negative bacillus, and has a monotric flagellum (Liu et al 2020; Effendi et al 2023). It is often found in fresh water and sewage. Usually considered a fish and amphibian pathogen, it is also involved in diarrheal disease in humans (Olusola et al 2020). The pathogen cells were isolated from the large intestine of humans, and found in some tissues. It is easy to grow on laboratory standard enteric media and produces hemolysis on blood agar. The sickness is caused by heat-labile enterotoxins and has a 12 to 48-hour incubation period. *A. hydrophila* can grow in a wide variety of temperatures, including as low as 0^{o}C (Figueras et al 2007; Monaghan et al 2008). This study aimed to determine the group of bioactive compounds contained in seaweed *E. spinosum* extract and its antibacterial activity against *V. alginolyticus and A. hydrophila.*

Material and Method

Place and time of the study. This research was carried out from March to May 2021. Samples of *E. spinosum* were collected from Jang Island, District of Moro, Karimun Regency, Province of Riau Island, Indonesia. The analysis of samples was carried out in the Marine Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru, Indonesia.

Methodology and design of research. This study was an experimental method, using a completely randomized design (CRD) with 2 factors, namely *E. spinosum* extract concentration and the species of the pathogens. The first factor consisted of 6 levels, namely 12.5% (A1), 25% (A2), 50% (A3), and 100% (A4) of *E. spinosum* extract, negative experimental control (NEC; water without any extract) and positive experimental control (PEC; water containing chloramphenicol 10%). The second factor consisted of *V. alginolyticus* (B1) and *A. hydrophila* (B2) with 3 times replications.

Extraction of bioactive compounds from E. spinosum. The fresh samples (spinous branches) of *E. spinosum* were cleaned using clean water and then air-dried and sliced thinly. The slices were dried under sunshine and blended until smooth and then soaked (maceration) with ethanol solution (96%) for 24 hours. A filter paper (Whatman No. 42) was used to filter the solution. The first filter paper was also macerated in the same way for the rest of the time until it generated a clear-colored filter. The filtrate was collected and separated from the solvent using a rotating vacuum evaporator at 60°C until all of the solvents had evaporated, yielding a crude extract of *E. spinosum* (Syawal et al 2020).

Phytochemical analysis. All of the following phytochemical analyzes were referred to Mera et al (2019), Syawal et al (2020) and Syawal et al (2021).

Saponin detection. A total of 0.1 g of sample was placed in a beaker, followed by 10 mL of hot water addition and a 5-minute boiling. The filtrate was employed as a test solution after the mixture was filtered. After shaking for 10 seconds and leaving for 10 minutes, 1 mL of 2 M HCl was added to the filtrate in a closed test tube. The formation of a stable foam suggested the presence of saponins.

Detection of steroids and terpenoids. Firstly, the chloroform liquid was prepared, then poured into 2 holes of the drip plate and fanned to dry. Then concentrated anhydrous acetic acid and concentrated H_2SO_4 were added into the 2 holes of the drip plate. The presence of steroid compounds was indicated by the green color formation and the presence of terpenoids was indicated by the formation of purple color.

Detection of tannins. The algal filtrate was added with 3 drops of $FeCl_3$, and then a dark green or blue tint indicated the presence of tannin chemicals.

Flavonoid detection. A volume of 5 drops of concentrated Mg and HCl was added to the red seaweed extract filtrate and aggressively agitated until a layer formed. The presence of flavonoid chemicals in the positive sample was indicated by its reddish-yellow to red color.

Alkaloid detection. In a test tube, 0.05 g of filtrate sample was placed, followed by drops of H_2SO_4 and shaking until completely mixed. Then it was poured into a drip plate and dripped with Meyer's reagent as a result of viewing white precipitation, and Wagner's reagent was identified by the presence of a brown precipitate, while Dragendorff's reagent was identified by the presence of an orange precipitate. If there is a precipitate, the sample is said to be positive for alkaloids.

Antibacterial activity. Aseptically *E. spinosum* extracts solutions were prepared at concentrations of 12.5% (A1), 25% (A2), 50% (A3), and 100% (A4). The same procedures were also taken for NEC and PEC preparation by using distilled water and water containing 10% of chloramphenicol, respectively. A total of 20 sheets of Whatman filter paper 45 pore size cut into rounds were dipped in each of these liquids, allowed to saturate, and then air-dried. These sheets of paper were referred to as paper discs. The antibacterial activity against pathogenic bacteria (*V. alginolyticus* and *A. hydrophila*) was assessed using the paper disc diffusion agar method (Effendi et al 2020). Bacterial isolates that had been regenerated in nutritional broth were taken in large quantities (up to 50 mL) and disseminated over Mueller-Hinton agar (MHA) media using a glass rod. On the inoculated plates, paper discs dripped with *E. spinosum* extract at concentrations of 12.5%, 25%, 50%, and 100%, as well as positive and negative controls, were inserted and incubated for 24 hours. Using a caliper, the diameter of the clear zone around the paper disc was measured to determine the inhibition zones.

Data analysis. The data was analyzed using One Way Analysis of Variance (ANOVA) to assess the difference among the treatments.

Results

Phytochemical analysis. In this study, the analysis was carried out to determine the content of groups of bioactive compounds which are present in the *E. spinosum* extract. The presence of a precipitate and a change in the color of the extract to orange indicated positive results in the alkaloids analysis. This red algae extract was positive for flavonoids if a shift in the hue of the preparation to a reddish yellow showed. However, the extract did not contain tannin, steroids, saponin, and triterpenoid (Table 1).

Table 1

Phytochemical analysis of *E. spinosum* extracts

No.	*Chemical analysis*	*Results*
1	Alkaloid	+
2	Tannin	-
3	Flavonoid	+
4	Steroid	-
5	Terpenoid	-
6	Saponin	-

Antibacterial activity. Antibacterial assay was used to determine whether the extract inhibited the pathogenic bacteria. The antibacterial activity test against *V. alginolyticus* and *A. hydrophila* can be observed by the formation of a clear zone around the paper disc. The size of the inhibition zone indicated that the *E. spinosum* extract inhibited the pathogenic bacteria (Figure 1 and Figure 2). The average of the inhibition zone against *V. alginolyticus* ranged from 10.87 to 21.03 mm, while against *A. hydrophila* ranged from 2.37 to 25.33 mm. The highest inhibition against *V. alginolyticus* was obtained using 50% extract, while the lowest one was obtained with 12.5% extract. In *A. hydrophila*, the highest inhibition zone was obtained using a 50% extract, while the lowest one was obtained using a 100% extract. More detailed data are presented in Table 2.

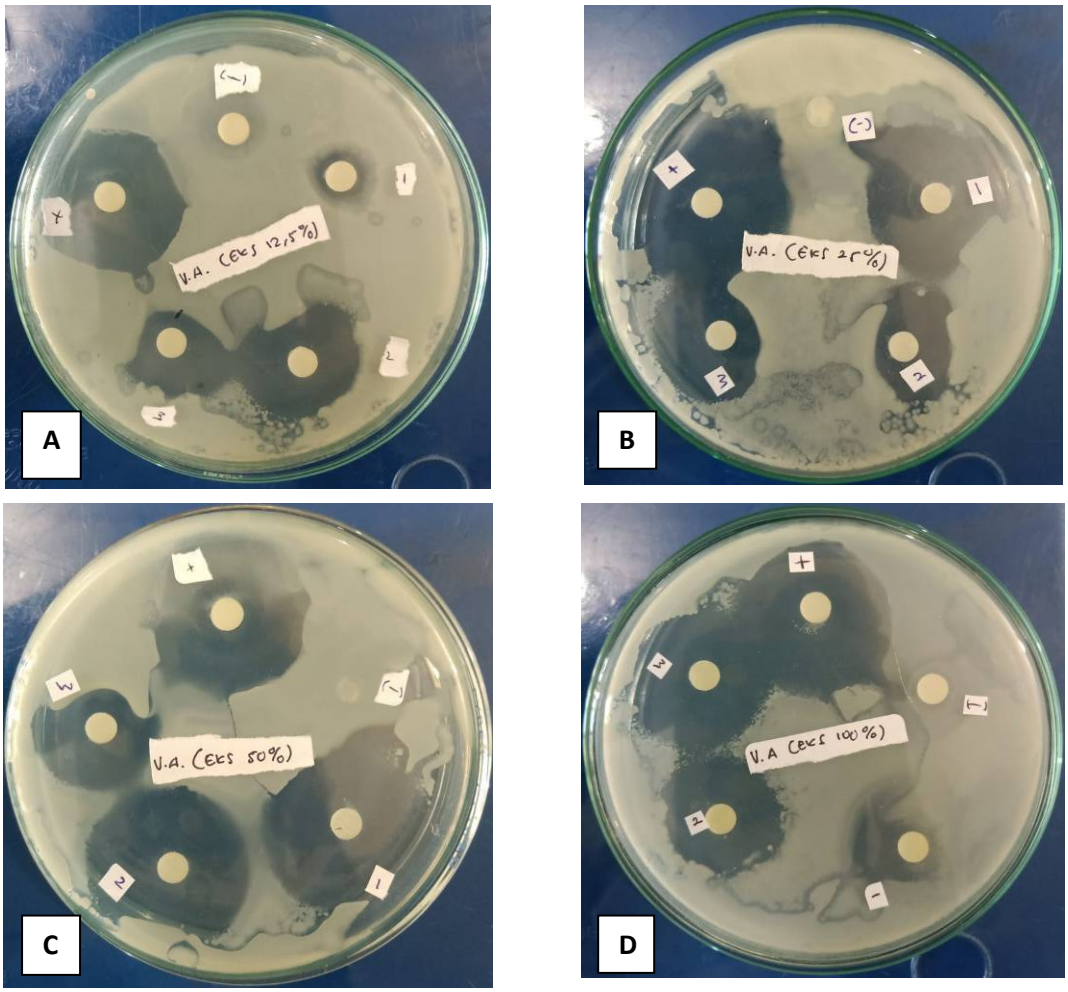

Figure 1. Inhibition zone to *V. alginolyticus*. A (12.5%), B (25%), C (50%) and D (100%).

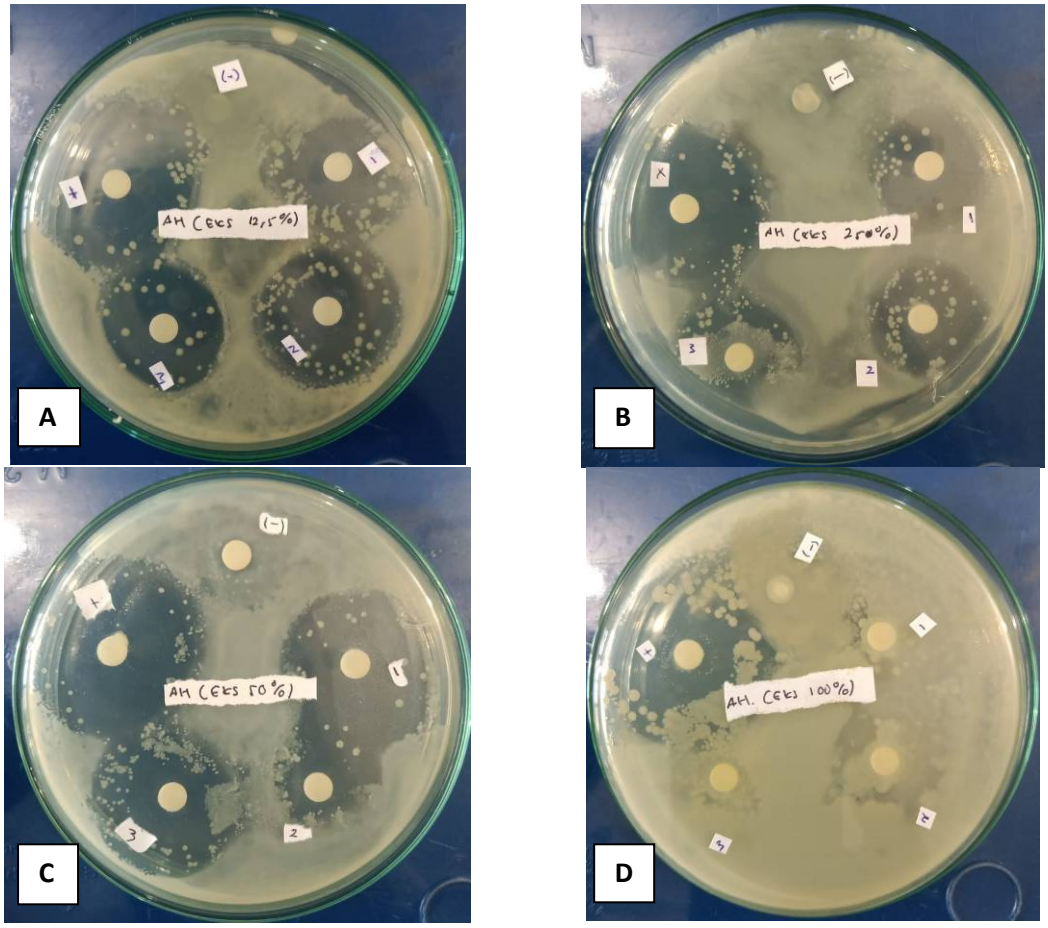

Figure 2. Inhibition zone to *A. hydrophila*. A (12.5%), B (25%), C (50%) and D (100%).

Table 2
Inhibition zone diameter of *E. spinosum* extract against *V. alginolyticus* and *A. hydrophila*

Bacteria	*Concentration (%)*	*R1*	*R2*	*R3*	*Average±SD (mm)*
V. alginolyticus	12.5 (A1 B1)	4.20	18.30	10.10	10.87±7.08
	25 (A2 B1)	22.60	10.10	14.60	15.77±6.33
	50 (A3 B1)	25.30	26.00	11.80	21.03±8.00
	100 (A4 B1)	7.50	17.10	21.60	15.4±7.20
	NEC B1	0.00	0.00	0.00	0.00±0.00
	PEC B1	23.20	25.20	24.30	24.23±1.00
A. hydrophila	12.5 (A1 B2)	17.20	16.70	13.80	15.9±1.84
	25 (A2 B2)	18.40	25.20	22.70	22.1±3.44
	50 (A3 B2)	26.10	24.2	25.70	25.33±1.00
	100 (A4 B2)	4.00	1.10	2.00	2.37±1.48
	NEC B2	0.00	0.00	0.00	0.00±0.00
	PEC B2	26.05	30.00	37.00	31.02±5.56

R = replication; SD = standard deviation; NEC = negative experimental control; PEC = positive experimental control.

Discussion. The results obtained in this study were not much different from those reported by some previous researchers. It revealed that the extract of *E. spinosum* contained only alkaloids and flavonoids. Safitri et al (2018) reported that the phytochemical analysis showed that alkaloids and saponins were the main components detected in the extract of the red algae. Brown sediment was formed with Wagner's reagent, yellow sediment with Mayer's reagent, and orange sediment with Dragendorff's reagent in alkaloids positive tests. When the extracts reacted with water and HCl, the presence of saponins was confirmed by the production of stable foam.

This plant is considered a source of bioactive compounds because it can produce various kinds of secondary metabolites with a wide range of biological activities, starting from antiviral activity, and antibacterial to antifungal. These bioactive substances have attracted the attention of the pharmaceutical world (Solomon & Santhi 2008). The cosmetic industry has utilized these substances for the production of ointments, soaps, lipsticks, lotions, shampoos, hair dyes, and creams. Some researchers are currently screening many natural antioxidants. This was carried out because it was alleged that the use of synthetic antioxidants has a carcinogenic effect (Nanditha & Prabhasankar 2009; Kong et al 2010; Effendi et al 2022). An antioxidant is used as a laxative material in the production of some pharmaceutical products and also in toothpaste and other dental products.

Safitri et al (2018) revealed that the extracts of *E. spinosum* at concentrations of 1%; 2%; 5%, and 10% (v/v) inhibited *S. aureus* of 1.98; 4.14; 7.42; and 10.27 mm, respectively. However, all red algae extracts failed to suppress the growth of *Bacillus subtilis*, *Salmonella enteritidis*, or *Aspergillus niger*, while extracts at 500 mg L^{-1} prevented the growth of *Staphylococcus aureus* (Rattaya et al 2015).

Some researchers (Neumann et al 2001; Effendi et al 2020) explained the mechanism of action of antimicrobial substances such as alkaloids and flavonoids against pathogenic bacteria. It is thought to inhibit the work of these bacterial enzymes, resulting in the disruption of metabolism or the death of these bacterial cells. It can also inhibit the formation of enzymes in the form of extracellular toxins which are virulence factors in bacteria. The mechanism of action of this antibacterial can also occur in several other ways (Babić et al 2019), namely damage to cell walls, changes in cell permeability, and inhibiting protein and nucleic acid synthesis. Many factors and circumstances can affect antibacterial work. Antibacterial compounds that attack bacteria will damage their cell walls or prevent their synthesis, so it will cause the formation of cells that are sensitive to osmotic pressure or known as trauma (Trombetta et al 2005).

Flavonoids are secondary metabolites of polyphenols and one of the most abundant natural compounds found in medicinal plant tissues. Flavonoids have been found to have antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, cardioprotective, anti-diabetic, anti-aging, and anticancer properties (Munhoz et al 2014; Marzouk 2016; Mathesius 2018; Sabri et al 2019). Many oxidation reactions, both enzymes and non-enzymes, are also inhibited by the flavonoid compound. The secondary metabolites are good reducing compounds, which will dissolve in polar solvents, such as methanol, ethanol, butanol, acetone, dimethylsulfoxide, and water. Flavonoid chemicals' mode of action permanently destroys cell membranes (Panche et al 2016).

Alkaloid compounds have antibacterial activity. The mode of action is by interfering with the peptidoglycan constituent of bacterial cells and causing the cell wall layer to not fully form. Disruption of peptidoglycan synthesis causes imperfect cell formation in which the cell wall only covers the cell membrane, resulting in cell death (Singh et al 2011).

The antimicrobial properties have been considered as an indicator that particular seaweed can synthesize bioactive secondary metabolites. Compounds with antiviral, anthelminthic, antifungal, and antibacterial properties have been detected in brown, red, and green algae (Kasanah et al 2015). The presence of secondary metabolites in algae is a form of adaptation to the environment. According to Franklin & Snow (2010), the mechanism of antimicrobial action can be achieved in four different ways. First is the inhibition of the synthesis or activation of enzymes that damage the bacterial cell wall, thus eliminating the ability to grow and causing lysis of the cell. The second is by direct action on the cell membrane by affecting cell permeability leading to leakage of intracellular compounds. The third is the disruption of the functioning of the bacterial ribosome, thereby inhibiting protein synthesis. Fourth is by interfering with the metabolism of nucleic acids leading to a loss of function of the synthesis of the cell.

The cell wall of Gram-positive bacteria mainly consists of peptidoglycans, teichoic acids, and plasma membranes Franklin & Snow (2010). Inhibition of the synthesis of cell wall bacteria can be through the destruction of peptidoglycans or teichoic acids (Silhavy et al 2010). Peptidoglycans consist of N-acetylglucosamines and N-acetylmuramic acids, and amino acids (Jankute et al 2015). The basic structure of peptidoglycans is a single

strand. Each strand of peptidoglycans is arranged side-by-side and connected by tetra-peptides crosslinking.

Amine groups in alkaloids from *E. spinosum* extracts can replace amine groups in tetra-peptides crosslinking. Since there are no carbonyl groups in alkaloids, peptide bonds will not be formed, and as a result, tetra-peptides crosslinking will be disconnected. In addition, saponins from *E. spinosum* extracts that contain –OH groups can form hydrogen bonds with oxygen in peptidoglycans. Strong interactions between H atoms in saponins and O atoms in peptidoglycans interfered with tetra-peptides crosslinking, as a result, the bonds within peptidoglycans are unstable and, subsequently, the peptidoglycans are easily destroyed. Another possible mechanism is the interference with teichoic acids (Brown et al 2013).

Conclusions. The ethanol extract of seaweed *E. spinosum* only contained two groups of secondary metabolites, namely alkaloids and flavonoids. These two substances act as an antibacterial against the growth of *V. alginolyticus* and *A. hydrophila*. The highest inhibition zone was for *V. alginolyticus* at a concentration of 50% and the lowest one was at a concentration of 12.5%. While for *A. hydrophila* the highest and the lowest inhibition zone were at a concentration of 50% and 100%, respectively. *E. spinosum* has been widely used as a raw material for the food industry. However, the presence of bioactive compounds makes it possible for this seaweed to be used as a source of antibacterial compounds in the future.

Acknowledgements. We take this opportunity to thank all colleagues in the Department of Marine Sciences who have participated in this study, and the Institute for Research and Community Service, University of Riau, Pekanbaru, Indonesia for funding this research.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Babić I., Bojić M., Maleš Z., Zadro R., Gojčeta K., Duka I., Rimac H., Jukić I., 2019 Influence of flavonoids' lipophilicity on platelet aggregation. Acta Pharmaceutica 69(4):607-619.

Brown S., Santa Maria J. P., Walker S., 2013 Wall teichoic acids of gram-positive bacteria. Annual Review of Microbiology 67:313-336.

Chen M. X., Li H. Y., Li G., Zheng T. L., 2011 Distribution of *Vibrio alginolyticus*-like species in Shenzhen coastal waters, China. Brazilian Journal of Microbiology 42: 884-896.

Damongilala L. J., Losung F., Dotulong V., 2021 [Antibacterial activity of fresh *Eucheuma spinosum* seaweed extract from the waters of Nain Island, North Sulawesi]. Jurnal Ilmiah Sains 21(1):91-95. [in Indonesian]

De Souza M. C. R., Marques C. T., Dore C. M. G., Da Silva F. R. F., Rocha H. A. O., Leite E. L., 2007 Antioxidant activities of sulfated polysaccharides from brown and red seaweeds. Journal of Applied Phycology 19(2):153-160.

Effendi I., Tanjung A., Sari D. M., 2020 Isolation of antibiotic-producing bacteria from extreme microhabitates in mangrove ecosystem. Journal of Physics: Conference Series 1655:012019.

Effendi I., Yoswaty D., Syawal H., Austin B., Lyndon A. R., Kurniawan R., Wahyuni S., Al-Harbi A., 2022 The use of medicinal herbs in aquaculture industry: a review. Current Aspects in Pharmaceutical Research and Development 7:7-20.

Effendi I., Rifai M., Nadira M. R., Syawal H., Suriani D. T., Yoswaty D., Riza S., 2023 Bioactivity of *Bruguiera gymnorrhiza* leaf extract on fish pathogenic bacteria *Staphylococcus aureus*, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa*. AACL Bioflux 16(1):89-98.

Figueras M. J., Aldea M. J., Fernandez N., Aspiroz C., Alperi A., Guarro J., 2007 *Aeromonas* hemolytic uremic syndrome. A case and a review of the literature. Diagnostic Microbiology and Infectious Disease 58(2):231-234.

Franklin T. J., Snow G. A., 2010 Biochemistry of microbial action. 2nd edition. London: Chapman and Hall Ltd., 224 pp.
Hijaz M. N., 2009 [Antioxidant activity test of carrageenan in red algae *Eucheuma spinosum* and *Gracillaria verrucosa*]. BSc thesis, Universitas Islam Negeri Malang, Malang, 62 pp. [in Indonesian]
Inayah N., Masruri M., 2021 Free-radical scavenging activity (FRSA) of secondary metabolite extracted from Indonesian *Eucheuma spinosum*. Alchemy: Journal of Chemistry 9(1):1-6.
Jankute M., Cox J. A. G., Harrison J., Besra G. S., 2015 Assembly of the mycobacterial cell wall. Annual Review of Microbiology 69:405-423.
Kasanah N., Triyanto, Seto D. S., Amelia W., Isnansetyo A., 2015 Antibacterial compounds from red seaweeds (Rhodophyta). Indonesian Journal of Chemistry 15(2):201-209.
Kong F., Zhang M., Liao S., Yu S., Chi J., Wei Z., 2010 Antioxidant activity of polysaccharide-enriched fractions extracted from pulp tissue of *Litchi chinensis* Sonn. Molecules 15(4):2152-2165.
Liao Y. C., Chang C. C., Nagarajan D., Chen C. Y., Chang J. S., 2021 Algae-derived hydrocolloids in foods: applications and health-related issues. Bioengineered 12(1): 3787-3801.
Liu J., Gao S., Dong Y., Lu C., Liu Y., 2020 Isolation and characterization of bacteriophages against virulent *Aeromonas hydrophila*. BMC Microbiology 20:141.
Manuel J., Cornelia M., Wijaya W., 2014 Utilization of *Eucheuma cottonii* and *Eucheuma spinosum* flour in narrow-barred Spanish mackerel meatballs. KnE Life Sciences 1: 12-18.
Marzouk M. M., 2016 Flavonoid constituents and cytotoxic activity of *Erucaria hispanica* (L.) Druce growing wild in Egypt. Arabian Journal of Chemistry 9(1):411-415.
Mathesius U., 2018 Flavonoid functions in plants and their interactions with other organisms. Plants 7(2):30.
Mera I. F. G., Falconí D. E. G., Córdova V. M., 2019 Secondary metabolites in plants: main classes, phytochemical analysis and pharmacological activities. Revista Bionatura 4(4):1000-1009.
Monaghan S. F., Anjaria D., Mohr A., Livingston D. H., 2008 Necrotizing fasciitis and sepsis caused by *Aeromonas hydrophila* after crush injury of the lower extremity. Surgical Infections 9(4):459-467.
Muawanah M., Ahmad A., Natsir H., 2016 Antioxidant activity and toxicity polysaccharide extract from red algae *Eucheuma cottonii* and *Eucheuma spinosum*. Marina Chimica Acta 17(2):15-23.
Munhoz V. M., Longhini R., Souza J. R. P., Zequi J. A. C., Leite Mello E. V. S., Lopes G. C., Mello J. C. P., 2014 Extraction of flavonoids from *Tagetes patula*: process optimization and screening for biological activity. Revista Brasileira de Farmacognosia 24:576-583.
Nanditha B., Prabhasankar P., 2009 Antioxidants in bakery products: a review. Critical Reviews in Food Science and Nutrition 49(1):1-27.
Narracci M., Acquaviva M. I., Cavallo R. A., 2013 Mar Piccolo of Taranto: *Vibrio* biodiversity in ecotoxicology approach. Environmental Science and Pollution Research International 21(3):2378-2385.
Ndobe S., Yasir I., Salanggon A. I. M., Wahyudi D., Ederyan, Muslihudin, Renol, Adel Y. S., Moore A. M., 2020 Eucheumatoid seaweed farming under global change - Tomini Bay seaweed trial indicates *Eucheuma denticulatum* (*spinosum*) could contribute to climate adaptation. AACL Bioflux 13(5):2452-2467.
Neumann N. F., Stafford J. L., Barreda D., Ainsworth A. J., Belosevic M., 2001 Antimicrobial mechanisms of fish phagocytes and their role in host defence. Developmental and Comparative Immunology 25(8-9):807-825.
Olusola S. E., Ajiwoju I. J., Emikpe B. O., 2020 Efficacy of tamarind *Tamarindus indica* leaves and mango *Mangifera indica* leaves as feed additives on growth, blood status and resistance to *Aeromonas hydrophila* in juvenile African catfish *Clarias gariepinus*. Croatian Journal of Fisheries 78(1):11-20.

Panche A. N., Diwan A. D., Chandra S. R., 2016 Flavonoids: an overview. Journal of Nutritional Science 5:e47.
Pereira C. S., Viana C. M., Rodrigues D. P., 2007 [Pathogenic vibrios in oysters (*Crassostrea rhizophorae*) served at restaurants in Rio de Janeiro: a public health warning]. Revista da Sociedade Brasileira de Medicina Tropical 40(3):300-303. [in Portuguese]
Rattaya S., Benjakul S., Prodpran T., 2015 Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, *Turbinaria ornata* and *Sargassum polycystum*, grown in Thailand. International Aquatic Research 7(1):1-16.
Sabri W. M. A. W., Yusop S. A. T. W., Sukairi A. H., Asaruddin M. R., 2019 Phytochemical screening, determination of antioxidant activity and alpha-amylase inhibitory activity of *Nypa fruticans* sugar. Materials Today: Proceedings 19(4):1745-1751.
Safitri A., Srihardyastutie A., Roosdiana A., Sutrisno, 2018 Antibacterial activity and phytochemical analysis of edible seaweed *Eucheuma spinosum* against *Staphyloccocus aureus*. The Journal of Pure and Applied Chemistry Research 7(3): 308-315.
Schets F. M., Van Den Berg H. H. J. L., Marchese A., Garbom S., De Roda Husman A. M., 2011 Potentially human pathogenic vibrios in marine and fresh bathing waters related to environmental conditions and disease outcome. International Journal of Hygiene and Environmental Health 214(5):399-406.
Silhavy T. J, Kahne D., Walker S., 2010 The bacterial cell envelope. Cold Spring Harbor Perspectives in Biolology 2(5):a000414.
Singh S., Swapnil, Verma S. K., 2011 Antibacterial properties of alkaloid rich fractions obtained from various parts of *Prosopis juliflora*. International Journal of Pharma Sciences and Research 2(3):114-120.
Solomon R. D. J., Santhi V. S., 2008 Purification of bioactive natural product against human microbial pathogens from marine seaweed *Dictyota acutiloba* J. Ag. World Journal of Microbiology and Biotechnology 24:1747-1752.
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.
Syawal H., Siregar A. R., Yuharmen Y., Effendi I., 2021 Phytochemical analysis and inhibitory effect of *Bruguiera gymnorrhiza* leaf extract on *Aeromonas salmonicida*, *Aeromonas hydrophila*, and *Edwardsiella ictaluri*. AACL Bioflux 14(4):1926-1933.
Trombetta D., Castelli F., Sarpietro M. G., Venuti V., Cristani M., Daniele C., Saija A., Mazzanti G., Bisignano G., 2005 Mechanisms of antibacterial action of three monoterpenes. Antimicrobial Agents and Chemotherapy 49(6):2474-2478.
Wei L. S., Wendy W., 2012 Characterization of *Vibrio alginolyticus* isolated from white leg shrimp (*Litopenaeus vannamei*) with emphasis on its antibiogram and heavy metal resistance pattern. Veterinarski Arhiv 82(2):221-227.
Wijayanto D., Bambang A. N., Nugroho R. A., Kurohman F., 2020 The growth model of *Eucheuma cottonii* cultivated in Karimunjawa Islands, Indonesia. AACL Bioflux 13(5):2551-2557.

Received: 24 November 2022. Accepted: 15 January 2023. Published online: 19 April 2023.
Authors:
Irwan Effendi, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru 28295, Indonesia, e-mail: helpingirwan@gmail.com
Muhammad Ridho Prayogi, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru 28295, Indonesia, e-mail: muhridhoprayogi27@gmail.com
Aras Mulyadi, Faculty of Fisheries and Marine Sciences, University of Riau, Pekanbaru 28295, Indonesia, e-mail: aras_mulyadi@hotmail.com

How to cite this article:
Effendi I., Prayogi M. R., Mulyadi A., 2023 Antibacterial activity of *Eucheuma spinosum* extract against *Vibrio alginolyticus* and *Aeromonas hydrophila*. AACL Bioflux 16(2):1105-1113.