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Antibacterial and Cytotoxic Activity from Ethyl Acetate Extract of *Sargassum* from Kabung Island West Kalimantan

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Abstract: *Sargassum* belongs to *Phaeophyceae* is widely distributed in Indonesian waters, including Kabung Island. Seaweed has bioactive compounds that potential to be developed. In the health sector, one of the global problems that still increasing is the bacterial infection. This condition leads us to explore antibiotic from natural resources to overcome this problem. *Sargassum* grow abundantly in the waters of Kabung Island and this genus has not been utilized by local community. For this reason, the aim of this study is to evaluate the antibacterial and cytotoxic activity from ethyl acetate extract of *Sargassum* from Kabung Island, West Kalimantan. Antibacterial activity test of the ethyl acetate extract against E. coli and S. aureus was conducted using the diffusion method with concentration series of 100; 50; 25; 5; 1; and 0.1 g/mL. While, the assessment of cytotoxic activity was carried out with concentration of 10; 100 and 1000 g/mL. The result showed that ethyl acetate extract of *Sargassum* from Kabung Island only showed antibacterial activity against *E. coli* bacteria. The greatest inhibition zone was 11.59 mm at a concentration of 100 g/mL, while in the cytotoxic test exhibited that the LC_{50} was more than 1000 ppm, so it was considered into non-toxic category.

Keywords: antibacterial; cytotoxic; ethyl acetate; sargassum; Kabung Island, West Kalimantan

Introduction

Macroalgae are one of the marine natural resources, responsible for primary productivity in the water environment (Sudhakar *et al.*, 2018). In marine waters, macroalgae have crucial roles in both of ecology and economic uses. Ecologically, macroalgae provide dissolved oxygen through photosynthesis process, food, and habitat for various aquatic organisms (John and Al-Thani, 2014). Furthermore, macroalgae possess an important economic value. They have been processed into several types of food and beverages. In general, based on the pigment content, macroalgae have been classified into three groups, such as *Chlorophyceae* (green algae), *Rhodophyceae* (red algae), and *Phaeophyceae* (brown algae) (Anggadiredja *et al*., 2006).

Sargassum belongs to *Phaeophyceae* (brown algae) because of the presence of fucoxantin pigment (Vidotti *et al*., 2014). *Sargassum* is widely distributed in West Kalimantan waters (Sofiana *et al*., 2022), including Kabung Island (Safitri *et al*., 2021; Sumarni *et al*., 2022). This genus grows abundantly in the coastal waters up to a depth of 20 meters. Seaweed has bioactive compounds that potential to be developed, such as alkaloids, glycosides, tannins and steroids (Sathya *et al*., 2017). These compounds have important roles with several biological activities, such as antiviral (Lomartire and Gonçalves, 2022), antibacterial (Warsidah *et al*., 2022), and

antioxidant (Lekameera *et al*., 2008). In addition, brown algae have been also reported to exhibit the potential of cytotoxic against cancer line (Khanavi *et al*., 2010; Monla *et al*., 2020; Sanger *et al*., 2021).

In the field of health, one of the global problems that still increasing is the bacterial infection. *Escherichia coli* and *Staphylococcus aureus* have been reported causing infectious disease. In the human body, *E. coli* for that matter is harmless. However, when the amount exceeds, this bacteria lead causing infection and inflammation (Szmolka and Nagy, 2013). According to previous study, *Sargassum* contains saponins, tannins, steroids, and flavonoid (Herawati and Pudjiastuti, 2021) that can be used as natural antibacterial agent. These bioactive compounds have been studied to inhibit several species of bacteria, such as *E. coli* and *Micrococcus luteus* (Sinurat *et al*., 2019), *Pseudomonas aeruginosa* (Siregar, 2012), *P. aeruginosa, S. aureus, Salmonella typhi*, and *Bacillus cereus* (Alamsjah *et al*., 2011).

Sargassum grow abundantly in the waters of Kabung Island. This genus has not been utilized optimally by local community. For this reason, the aim of this study is to evaluate the antibacterial and cytotoxic activity from ethyl acetate extract of *Sargassum* from Kabung Island, West Kalimantan.

Methods

Samples Collection and Identifications

Samples of *Sargassum* were collected from Kabung Island waters, Bengkayang Regency, West Kalimantan in June 2020. Samples identification, preparation, extraction, and test of cytotoxic activity were carried out in the Laboratory of Marine Science, Faculty of Mathematics and Natural Sciences, Universitas Tanjungpura. Furthermore, for the test of antibacterial activity was conducted in the Laboratory of Penerapan Mutu Hasil Perikanan (UPT-PMHP) West Kalimantan Province.

Samples Preparation and Extractions

Samples were cleaned from sand and epiphytes using seawater, then rinsed with clean water. After that, samples were stored in cooling box. The clean *Sargassum* samples were then cut into small pieces and dried at room temperature. Samples extraction was done using the maceration method. A total of 500 g dried samples was macerated with ethyl acetate solvent for 48 hours. The macerate was then filtered and evaporated to obtain a crude extract of ethyl acetate.

The test of Antibacterial Activity

Antibacterial activity test of the ethyl acetate extract was conducted using the diffusion method. Isolates from the test bacteria *E. coli* and *S. aureus* were grown in Nutrient Agar (NA) media for 48 hours. Samples were made with a concentration series of 100; 50; 25; 5; 1; and 0.1 g/mL. A total of 50 µL of each concentration was put into a diffusion well in NA medium which had been inoculated with the test bacteria. The positive control used chloramphenicol, and the negative control used ethyl acetate as the extract solvent. Observations were made by observing the formation of inhibition zone for 24 hours (Alamsjah *et al.*, 2014).

The Assessment of Cytotoxic Activity

The assessment of cytotoxic activity of the ethyl acetate extract of *Sargassum* was carried out by preparing an extract solution with a concentration series of 10; 100 and 1000 g/mL. *Artemia salina* were grown for 48 hours using sea water. *A. salina* 48 hours old was taken as many as 10 individuals, put into a vial bottle, then added seawater to reach final volume of 5 mL. The observations were made after 24 hours by counting live *A. salina*. The test was done with three replications, then the percentage of death *A. salina* was calculated using the formula as follows:

$$\% \text{ mortality} = \frac{number\ of\ dead\ larvae}{total\ number\ of\ larvae}\ x\ 100\%$$

The determination of the LC_{50} was done using the probit analysis method. An extract was classified to be toxic if the LC_{50} value is ≤1000 µg/mL and non-toxic if the LC_{50} value is ≥1000 µg/mL (Baud *et al*., 2014).

Result and Discussion

Antibacterial Activity

Samples of *Sargassum* in this study were taken from Kabung Island waters, West Kalimantan. The sample was dried at room

temperature, to protect the evaporation of the active substance from the samples. Thus, the aim of the sample drying was to expand the sample surface. So that, when samples were macerated, the interaction between samples and the solvent was greater and the extraction process was running optimally (Handayani and Nurcahayanti, 2015; Adam *et al*., 2019).

In this study, the yield of ethyl acetate extract was 0.0634%. Extract yield showed the total secondary metabolites extracted from the samples. The yield quantity depended on the total moisture content of the samples. The less water content, the greater the yield obtained (Naina *et al*., 2019). According to previous study, ethyl acetate extract of *S. cinereum* showed the percentage of the yield was 0.47% (Alamsjah *et al*., 2014), and *S. polycystum* had the yield percentage as much as 0.91% (Savitri *et al*., 2017). The concentrated ethyl acetate extract was a paste form and showed dark green in color. Sidauruk *et al*. (2021) stated that the brownish green color of concentrated extract of *S. plagyophyllum* was due to the presence of pigments such as fucoxanthin, carotenoids, chlorophyll, and xanthophylls which are dominant in macroalgae.

Ethyl acetate is classified into semi polar organic solvent. This solvent is able to dissolve a large number of compounds from the samples. The use of ethyl acetate solvent is easier to evaporate, not hygroscopic and has low toxicity (Lidiawati *et al*., 2018). In the present study, antibacterial activity test from ethyl acetate extract of *Sargassum* from Kabung Island only showed the inhibitory activity toward *E. coli* (Table 1). The concentration of 100 µg/mL exhibited the highest value of inhibition zone. The potential of inhibition can be influenced by different types of test bacteria (Muharani *et al*., 2017).

Table 1. Diameter of inhibition zone of antibacterial activity from the ethyl acetate extract of *Sargassum*

Concentration (µg/mL)	Diameter of inhibition zone (mm)	
	E. coli	***S. aureus***
100	11.59	-
50	5.32	-
25	5.49	-
5	-	-
1	-	-
0,1	-	-
Positive control	13.84	14.11
Negative control	-	-

There are differences in the structure of the cell wall between *E. coli* and *S. aureus*. *E. coli* belongs to Gram-negative whose cell walls consist of one or more thin layers of peptidoglycan, while *S. aureus* is Gram-positive bacteria with a thick and rigid peptidoglycan layer (Rastina *et al*., 2015). The potential of the extract in inhibiting the growth of the bacteria depends on its ability to penetrate the bacterial cell wall. In addition, the yield value (0.0634%) showed that the bioactive compound in the extract is very low. Therefore, these compounds did not have the ability to penetrate into the cell wall of the *S. aureus*. In the test of antibacterial activity, the inhibition zone is influenced by the extract concentration, the metabolites content in the extract, the diffusion power of the extract, as well as the type of test bacteria.

Cytotoxic Activity

In this study, the assessment of cytotoxic activity from the ethyl acetate extract of *Sargassum* was conducted by the Brine Shrimp Lethality Test (BSLT) method, using *A. salina* larvae aged 48 hours. The BSLT method is simple, easy, and has of 95% confidence level. The liquid extract diffused through the thin *A. salina* membrane, and if the extract contains the toxic compounds, the larvae will die (Muaja *et al*., 2013). The result of the LC_{50} value showed that the ethyl acetate extract of *Sargassum* from Kabung Island waters was non-toxic category (Table 2). Baud *et al*. (2014) stated that the LC_{50} value is ≥1000 µg/mL was classified into non-toxic category (Baud *et al*., 2014).

Table 2. Cytotoxic test from Ethyl Acetate Extract of *Sargassum*

Concentration (µg/mL)	Mortality (%)	Regression equation	LC_{50} (µg/mL)	Toxicity category
10	20	y = 0,375x + 3,7433		
100	26.67	R^2 = 0,9461	2244	Non toxic
1000	46.67			

Conclusion

The ethyl acetate extract of *Sargassum* from Kabung Island waters only showed antibacterial activity against *E. coli* bacteria. The greatest inhibition zone was 11.59 mm at a concentration of 100 g/mL, while in the cytotoxic test exhibited that the LC_{50} was more than 1000 ppm, so it was considered into non-toxic category.

References

Adam, N., Lolo, W.A., Sudewi, S. (2019). Aktivitas Antibakteri Fraksi Alga *Turbinaria ornata* (Turner) J. Agardh yang Diperoleh dari Perairan Teluk Manado. *J. Pharmacon*, 8(2): 325-334. DOI: https://doi.org/10.35799/pha.8.2019.29298

Alamsjah, M.A., Nurhayati, D., Tjahjaningsih. (2011). Pengaruh Ekstrak Alga Cokelat (*Sargassum* sp.) terhadap Pertumbuhan Bakteri Escherichia coli Secara in Vitro. *J. Ilmiah Perikanan dan Kelautan*, 3(2): 69-78. DOI: 10.20473/jipk.v3i1.11627

Anggadiredja, J.T.; Zatnika, A.; Purwoto, H.; dan Istini, S. (2008). *Rumput Laut, Pembudidayaan, Pengolahan, dan Pemasaran Komoditas Perikanan Potensial*. Jakarta: Penebar Swadaya. 147 hlm.

Baud, Grace S., Meiske, S., Sangi, Harry, S.J., Koleangan. (2014). Analisis Senyawa Metabolit Sekunder dan Uji Toksisitas Ekstrak Etanol Batang Tanaman Patah Tulang (*Euphorbia tirucalli* L.) dengan Metode Brine Shrimp Lethality Test (BSLT). *J. Ilmiah Sains*., 14(2): 106-112. DOI: https://doi.org/10.35799/jis.14.2.2014.6065

Handayani, P.A. dan Nurcahyanti, H. (2015). Ekstraksi Minyak Atsiri Daun Zodia (*Evodia suaveolens*) dengan Metode Maserasi dan Distilasi Air. *J. Bahan Alam Terbarukan*. 4(1): 1-7. DOI: https://doi.org/10.15294/jbat.v3i1.3095

Herawati, D. and Pudjiastuti, P. (2021). Effect of Different Solvents on The Phytochemical Compounds of *Sargassum* sp. from Yogyakarta and East Nusa Tenggara. *Journal of Physics: Conference Series*, 1783: 012001. DOI 10.1088/1742-6596/1783/1/012001

John, D.M. and Al-Thani, R.F. (2014). Benthic marine algae of the Arabian Gulf: a critical review and analysis of distribution and diversity patterns. *Nova Hedwigia*, 98(3-4): 341-392. DOI:10.1127/0029-5035/2014/0156

Khanavi, M., Nabavi, M., Sadati, N., Ardekani, M.S., Sohrabipour, J., Nabavi, S.M.B., Ghaeli, P., Ostad, S.N. (2010). Cytotoxic activity of some marine brown algae against cancer cell lines. *Biol. Res*., 43: 31-37. DOI: doi.org/10.4067/S0716-97602010000 100005

Lekameera, Vijayabaskar, R.P., Somasundaram, S.T. (2008). Evaluating antioxidant property of brown ALGA *Colpomenia sinuosa* (DERB. ET SOL). *African Journal of Food Science*, 2: 126-130. https://www.researchgate.net/publication/237740016

Lidiawati, T.E., Saleh, C., Alimuddin. (2018). Sintesis Etil Asetat dari Hasil Fermentasi Kulit Singkong (*Manihot esculenta* L.) dengan Asam Asetat Menggunakan Katalis Asam. *Prosoding Seminar Nasional Kimia*, 82-86. URL: http://jurnal.kimia.fmipa.unmul.ac.id/index.php/prosiding/article/view/764>.

Lomartire, S. and Gonçalves, A.M.M. (2022). Antiviral Activity and Mechanisms of Seaweeds Bioactive Compounds on

Enveloped Viruses - A Review. *Mar. Drugs*., 20: 385. DOI: 10.3390/ md20060385.

Monla, R.A., Dassouki, Z., Kouzayha, A., Salma, Y., Gali-Muhtasib, H., Mawlawi, H. (2020). The Cytotoxic and Apoptotic Effects of the Brown Algae *Colpomenia sinuosa* are Mediated by the Generation of Reactive Oxygen Species. *Molecule*, 25: 1993. DOI: 10.3390/molecules25081993

Muaja, A.D., Koleangan, H.S.J., Runtuwene, M.R.J. (2013). Uji Toksisitas dengan Metode BSLT dan Analisi Kandungan Fitokimia Ekstrak Daun Soyogik (*Saurauia bracteosa* DC) dengan Metode Soxhletasi. *J. MIPA Unsarat*, 2(2): 115-118. DOI: doi.org/10.35799/jm.2.2.2013.3000

Muharani, Fitrya, Farida, S. (2017). Uji Aktivias Antibakteri Ekstrak Etanol Tanaman Obat Suku Musi di Kabupaten Musi Bayuasin Sumatera Selatan. *J. Kefarmasian Indonesia*, 7(2): 127-135. DOI: https://doi.org/10.22435/jki.v7i2.3493

Naina, Y., Wulandari, R., Raza'i, T.S. (2019). Skrining Komponen Bioaktif Ethanol 96% *Sargassum* sp. sebagai Antibakteri terhadap *Vibrio harveyi*. *J. Intek Akuakultur*, 3(2): 22-33. DOI:10.31629/ INTEK.V3I2.1360

Rastina, Sudarwanto, M., Wientarsih, I. (2015). Aktivitas Antibakteri Ekstrak Etanol Daun Kari (*Murraya koenigii*) terhadap *Staphylococcus aureus*, *Escherichia coli*, dan *Pseudomonas* sp. *J. Kedokteran Hewan*, 9(2): 185-188. DOI: doi.org/10.21157/ j.ked.hewan.v9i2.2842

Safitri, I., Warsidah, Sofiana, M.S.J., Kushadiwijayanto, A.A., Sumarni, T.N. (2021). Total Phenolic Content, Antioxidant and Antibacterial Activities of *Sargassum polycystum* of Ethanol Extract from Waters of Kabung Island. *BERKALA SAINSTEK*, 9(3): 139-145. DOI: https://doi.org/10.19184/bst.v9i3.27199

Sanger, G., Rarung, L.K., Wonggo, D., Dotulong, V., Damongilala, L.J., Tallei, T.E. (2021). Cytotoxic activity of seaweeds from North Sulawesi marine waters against cervical cancer. *Journal of Applied Pharmaceutical Science*, 11(09): 066-073. DOI: 10.7324/JAPS.2021.1109 08

Sathya, R., Kanaga, N., Sankar, P., Jeeva, S. (2017). Antioxidant Properties of Phlorotanninsfrom Brown Seaweed *Cystoseira trinodis* (Forsskal) C. Agardh. *Arabian J. Chem*., 10: 2608-2614. DOI:10.1016/j.arabjc.2013.09.039

Savitri, I., Suhendra, L., Wartini, N.D. (2017). Pengaruh Jenis Pelarut pada Metode Maserasi terhadap Karakteristik Ekstrak *Sargassum polycatum*. *J. Rekayasa dan Manajemen Agroindustri*. 5(3): 93-101. https://ojs.unud.ac.id/index.php/jtip/article/view/35504

Sidauruk, S.W., Sari, N.I., Diharmi, A., Arif, I. (2021). Aktivitas Antibakteri Ekstrak *Sargassum plagyophyllum* terhadap Bakteri *Listeria monocytogenes* dan *Pseudomonas aeruginosa*. *J. PHPI*., 24(1): 27-37. DOI: https://doi.org/10.17844/ jphpi.v24i1.33417

Sinurat, A.A.P., Renta, P.P., Herliany, N.E., Negara, B.F., Purnama, D. (2019). Uji Aktivitas Aantibakteri Ekstrak Metanol Rumbut Laut *Gracilaria edulis* terhadap Bakteri *Aeromonas hydrophil*. *J.Enggano*, 4(1): 105-114. DOI: https://doi.org/10.31186/ jenggano.4.1.105-114

Siregar, A.F., Sabdono, A., Pringgenies, D. (2012). Potensi Antibakteri Ekstrak Rumput Laut terhadap Bakteri Penyakit Kulit *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* dan *Micrococcus inteus*. *Journal of Marine Research*, 1(2): 152-160. DOI: https://doi.org/10.14710/jmr.v1i2.2032

Sofiana, M.S.J., Nurrahman, Y.A., Warsidah, Minsas, S., Yuliono, A., Safitri, I., Helena, S., Risko. (2022). Community Structure of Macroalgae in Lemukutan Island Waters, West Kalimantan. *Jurnal Ilmu Kelautan SPERMONDE*, 8(1): 1-8. DOI: https://doi.org/10.20956/jiks.v8i1.17914

Sudhakar, K., Mamat, R., Samykano, M., Azmi, W.H., Ishak, W.F.W., Yusaf, T. (2018). An overview of marine macroalgae as bioresource. *Renewable and Sustainable Energy Reviews*, 91: 165-179. DOI: https://doi.org/10.1016/j.rser.2018.03.100

Sumarni, T.N., Warsidah, Safitri, I., Kushadiwijayanto, A.A., Sofiana, M.S.J. (2022). Analisis Kandungan Proksimat dan Mineral Zink dari *Sargassum* sp. asal Perairan Pulau Kabung. *Oseanologia*, 1(1): 24-27. DOI: doi.org/10.26418/jose.v1i1. 53467

Szmolka, A. and Nagy, B. (2013). Multidrug resistant commensal Escherichia coli in animals and its impact for public health. *Frontier in Microbiology*, 4(258): 1-13. DOI: doi: 10.3389/fmicb.2013.00258

Vidotti, E.C., Rollemberg, M., Do, C.E. (2014). Algas: Da economia nos ambientes aquáticos à biorremediação e àquímica analítica. *Quim. Nova*, 27: 139-145. DOI: https://doi.org/10.1590/S0100-40422004 000100024

Warsidah, Safitri, I., Sofiana, M.S.J., Helena, S. (2022). Antibacterial Activity from Ethanol and Ethyl Acetate Extracts of *Padina pavonica* Hauck from Kabung Island against *Escherichia coli*. *Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology*, 18(1): 1-6. DOI: https://doi.org/10.14710/ijfst.18.1.1-6