

Bioecology of *Sargassum* sp. and its Extract Bioactivity as Anti-MDR Bacteria

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Abstract

This study was done to investigate the bioecology and extract bioactivity of *Sargassum* sp from Jepara district. Seaweed was collected from Teluk Awur, Panjang Island, Bandengan, Ujung Piring and Bondo. There were seven species of *Sargassum* identified i.e. *S. duplicatum*, *S. polycystum*, *S. echinocarpum*, *S. cinerium*, *S. crassifolium*, *S. plagyophillum* and *S. binderi*. Community structure indices were calculated based on data collected by survey. Antibacterial activity of *Sargassum* extract was done on MDR bacteria. The results showed that diversity index at Panjang Island, Bandengan, and Ujung Piring were categorized as medium, while Teluk Awur and Bondo were categorized as low. Evenness index was high at Teluk Awur, Panjang Island, Bandengan and Ujung Piring and Bondo was low. There was no dominant species found in most of the locations. The principal component analysis exhibited the main variable that affected the *Sargassum* at Teluk Awur was phosphate, Panjang Island was depth, Ujung Piring was salinity and Bondo was temperature, dissolved oxygen and pH, respectively. There was character similarity between Panjang Island and Bandengan (99.73%), meanwhile, at Teluk Awur, Ujung Piring, and Bondo, the similarity was 99.62%, 99.60%, and 99.52%, respectively. Antibacterial activity of *Sargassum* sp. demonstrated a positive activity against the MDR bacteria, *Staphylococcus aureus* (6.28±0.04 mm), *Escherichia coli* (6.96±0.19 mm), and *S. epidermidis* (12.65±0.23 mm). Clustering analysis of extracts with bactericidal characteristic found three classes with similarity of class 1, 2 and 3 at 72.33%, 76.80% and 58.50%, respectively. *Sargassum* sp. was categorized in class 1 had the best antibacterial activity and the highest abundance value.

Keywords: ecology, community, *Sargassum* sp., extracts, antibacterial

Introduction

Research on seaweed, *Sargassum* sp., has been conducted quite extensively but information that explains about environment towards distribution, diversity, and bioactive abundance from *Sargassum* sp. is limited. *Sargassum* sp. has been reported to show promising pharmacological properties serving as antiviral (Hardouin et al., 2013), antitumor (Nianjun et al., 2004), antioxidant (Rastian et al., 2007; Bhaigyabati, 2011; Zaragoza et al., 2008; Khaled et al., 2012; Tanniou et al., 2013), antifouling (Plouguerne et al., 2010), and antifungal (Manivannan, 2011; Khaled et al., 2012). *S. fulvellum* and *S. thunbergii* showed antipyretic, analgesic, and anti-inflammatory activities in mice (Kang et al., 2008). *S. echinocarpum*, *S. duplicatum* and *S. polycystum* performed potential antioxidant (Widowati et al., 2014). *S. aquifolium*, *S. ilicifolium* and *S. polycystum* showed antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*

and *Escherichia coli* (Puspita et al., 2012). *S. pallidum* can serve as antioxidant and anti-hemolysis agents exhibiting potential for further exploration as functional food or complementary medicine (Bhaigyabati et al., 2011; Li et al., 2017). *S. tenerrimum* from India is reported to have antibacterial activity against *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus* sp., *Streptococcus* sp., *Pseudomonas aeruginosa*, *Salmonella* sp., *Shewanella* sp., *Vibrio fluvialis*, *V. splendidus*, *V. cholera*, *V. parahaemolyticus*, *Shigella flaxneri*, *Staphylococcus epidermidis*, *Aeromonas liquefaciens* and *Bacillus subtilis*. Furthermore, *Sargassum* sp. also have antifungal activity against *Aspergillus niger*, *A. flavus*, *A. strettus*, *Candida albicans* and *Penicillium* sp. (Manivannan et al., 2011).

The above research focuses only on the perspective of active compounds, but which relates to ecological factors is very limited. This study was

conducted to see the bioactive compounds from *Sargassum* sp. and its relation to ecological conditions in Jepara. This is considering that the area is one of the locations that have seaweed resources in Indonesia. One of the most abundant seaweed is *Sargassum* sp.

Exploration of *Sargassum* sp. especially about the use of active compounds is necessary in order to be utilized. In addition, information about the relationship between *Sargassum* sp. and the environment in producing secondary metabolites can provide direction to the sustainable management of these marine resources.

Materials and Methods

Observation of *Sargassum* sp.

Sargassum were collected from 5 locations in Jepara, i.e. Teluk Awur, Panjang Island, Bandengan, Ujung Piring and Bondo. Observation of *Sargassum* sp. was conducted by 100 m line transect (English *et al.*, 1997). Environmental parameters observed were temperature, brightness, depth, salinity, pH, DO and current velocity. A whole thallus of seaweed was taken for taxonomic identification based on morphology study. Collected seaweeds were rinsed with tap water to remove the epiphytes and remaining debris. Prior to natural drying under the shade, seaweeds were cut to pieces (± 5 cm). Further, the dried seaweeds were ground by means of with multiuse blender to obtain powder material (Manivannan *et al.*, 2011).

Extraction of *Sargassum* sp.

Twenty five grams of dry seaweed was extracted gradually by using 100 mL n-hexane, ethyl acetate, and methanol. The extraction was performed for 24 hours in room temperature. The extracted seaweed was filtered through Whatman filter paper. The remaining residues of seaweed were re-extracted applying the same process and solvents. Filtrates obtained from the extraction process were pooled. N-hexane, ethyl acetate and methanol filtrates were evaporated to dryness with vacuum rotary evaporator at 40 °C in 500 mmHg. N-hexane, ethyl acetate and methanol extracts of *Sargassum* sp. were dried using N₂ prior to storage in -20 °C (Kanjana *et al.*, 2011).

Antibacterial activity test

Antibacterial activity test used agar diffusion method with Zobell media (peptone, yeast, and bacto agar). Antibacterial test used 3 isolates of MDR candidates, i.e. *Staphylococcus aureus*, *Esherichia coli*, and *S. epidermidis*.

Bacteria culture was centrifuged at 5000 rpm for 10 minutes then dissolved with PBS and measured using Optical Density Spectrophotometer λ 600 nm between 0.6–0.8 (Lalitha, 2004) then pipetted for 0.1 mL and inoculated to the surface of media petri, after that it was incubated for 30 minutes. Antibacterial test using extract solution and antibiotic chloramphenicol as the control (+) for with concentration of 2500 ppm and in each paper disc sterile was given extract solution for 20 μ l (50 μ g.disc⁻¹) (Kanjana *et al.*, 2011). Each paper disc was placed in petri dish with agar and bacteria then being storage in the incubator with the temperature of 37°C for 24-48 h (Pelczar *et al.*, 2005).

Data analysis

The abundance value was used to describe the community structure of *Sargassum* which includes Diversity Index (H'), Evenness Index (e) and Dominancy Index (C). Multivariable statistic approach was used to analyze physical-chemical water parameters. This analysis aimed to see the linkages of physical-chemical variables towards observation stations based on the approach of analysis to the main components (OCA).

Results and Discussion

This study found 3 species of *Sargassum* in Teluk Awur, 4 species in Panjang Island, 3 species in Bandengan, 3 species in Ujung Piring, and 1 species in Bondo. Highest total abundance was found in Bondo for 1510 ind.200m⁻². Highest Diversity Index (H') was in Panjang Island (1.24), Bandengan and Ujung Piring (1.09– moderate), Teluk Awur (1.00 – low) and Bondo (0.00 – low).

The value of Evenness Index was similar and categorized as high at all locations except Bondo, i.e. Teluk Awur (0.91), Panjang Island (0.90), Bandengan (0.99h), and Ujung Piring (0.99) which shows that the environmental is stable. While in Bondo was low (0.00) and shows that environment is under pressured and unstable. It is probably caused by the existence of PLTU (Steam Electricity Power Plant) activity. There is no species domination in Teluk Awur, Panjang Island and Bandengan. *S. binderi* dominates Bondo area, probably due to highest current velocity than the other stations. Current has a role to clean up the dirt attached so that the nutrient can go to the entire surface of thallus body through diffusion osmosis process.

Result of principle component analysis (F₁x F₂) shows, the main component of variables of water quality in Teluk Awur was phosphate. It is assumed that in the land area of that station there is an agricultural activity that flows solved phosphate

waste and carried to observation station. Panjang Island with the depth because this location is a land that separated from the mainland. Ujung Piring with salinity, it is assumed because the beach is sloping/calm and enclosed by the bay so the evaporation process is high. Salinity at Ujung Piring is assumed low/high. It is because of the beach sloping/calm and enclosed by the bay, thus evaporation process is high. Bondo with

temperature, DO and pH, it is assumed because of outcome from waste of PLTU activity result and it causes high temperature and increasing condition of DO and pH, meanwhile Bandengan has no main component (Figure 1). Result of correlation Pearson test of water quality towards the abundance shows, each station has different amount and variable composition which influenced to the abundance distribution. Teluk Awur has 8 variables; Panjang

Table 1. The Abundance Value (ind.200m⁻²), Diversity Index (H'), Evenness Index (e), Dominancy Index (C) and The Average Value of Water Parameters

Species	Station				
	Teluk Awur	Panjang Island	Bandengan	Ujung Piring	Bondo
<i>S. duplicatum</i>	504	147	0	256	0
<i>S. polycystum</i>	159	142	0	299	0
<i>S. echinocarpum</i>	437	0	258	0	0
<i>S. cinereum</i>	0	75	329	0	0
<i>S. crassifolium</i>	0	337	0	0	0
<i>S. plagyophyllum</i>	0	0	222	0	0
<i>S. binderi</i>	0	0	0	368	1510
Total Abundance (ind.200 m ⁻²)	1100	701	809	923	1510
Total Species	3	4	3	3	1
Diversity Index (H')	1 Low	1,24 Medium	1,09 Medium	1,09 Medium	0 Low
Index of Evenness (e)	0.91 High	0.90 High	0.99 High	0.99 High	0 Low
Dominancy Index (C)	0.39 -	0.33 -	0.34 -	0.34 -	1 +
Depth (m)	1.2	3.9	2.8	0.9	3.6
Visibility (m)	1.2	2.3	2.1	0.8	1.5
DO	4.1	5.6	5.8	5.4	5.8
pH	7.2	7.2	7.2	7.2	7.3
Temperature (°C)	31	31	30	30	32
Salinity (‰)	32	31	31	32	31
Current (m.minute ⁻¹)	6.0	5.8	8.1	1.2	8.2
PO ₄ (ppm)	0.013	0.003	0.006	0.001	0.001
Nitrate (ppm)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005

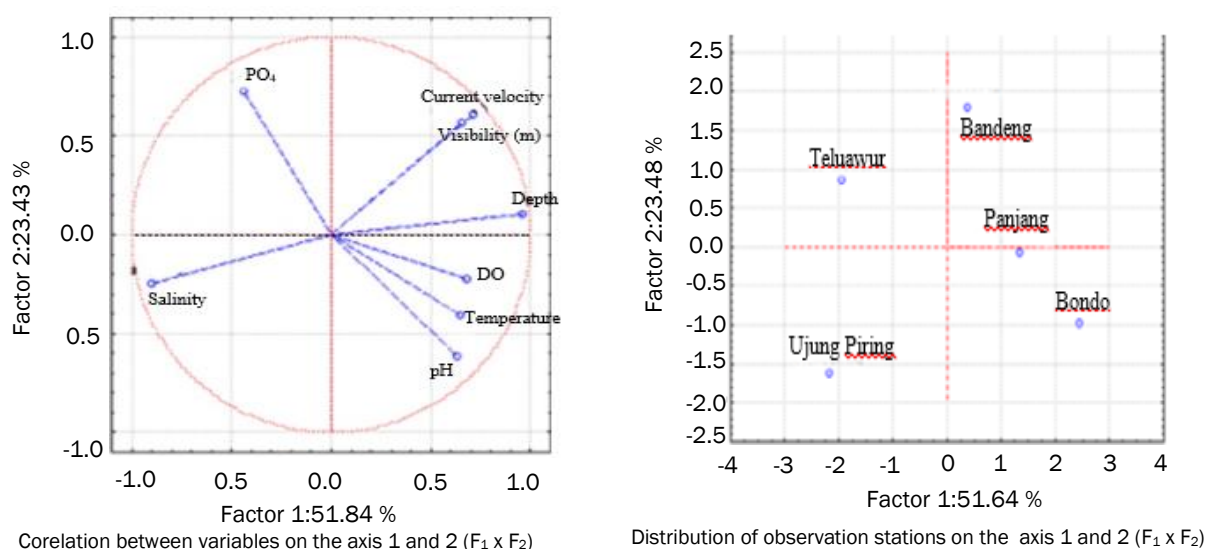


Figure 1. Correlation Between Variables on the Axis 1 and 2 (F₁ x F₂) and Distribution of Observation Stations on the Axis 1 and 2 (F₁ x F₂)

Island and Bandengan have 7 variables with stable temperature and salinity, Ujung Piring has 4 variables and variable pH, temperature and phosphate tend to be stable. Bondo has 7 variables and stable temperature variable. The result of correlation test shows that there is a variable difference which has positive and negative correlation towards the abundance in each station.

Regression multivariable equation shows the linkage of water parameters towards the distribution of abundance in each station. Analysis result between water qualities towards abundance results the equation that has same component, which is phosphate. The component in Teluk Awur is pH (+) and Phosphate (-) which function as antagonist in influencing the abundance. It shows, that higher pH value until certain limits causes increase in distribution of Sargassum abundance in Teluk Awur, in the contrary, higher phosphate results the decrease in abundance distribution.

Regression multivariable equation of water quality characteristics towards abundance, components in Panjang Island are Current (-) and Phosphate (+). It has a function as antagonists in influencing the abundance. It shows that the higher value of current causes the decrease of Sargassum distribution, while the higher value of phosphate will increase in distribution of its abundance.

Components in Bandengan are Depth (+) and Phosphate (-) that function as antagonist in influencing the abundance. It shows that deeper water causes the more abundant the distribution of Sargassum abundance, while higher Phosphate value will decrease the distribution of its abundance. Components in Ujung Piring are Current (-) and Phosphate (+) that function as synergy in pressing the abundance. It shows that stronger current higher value of phosphate, so distribution of Sargassum abundance decreases. Components in Bondo are DO (+) and Current (+) that function as synergy in the increasing of abundance. It shows that higher DO and current increase distribution of Sargassum.

Phosphates in Teluk Awur, Bandengan and Ujung Piring station have negative correlation because the value of phosphate is higher than in Panjang Island. Phosphate is limiting factor which is needed in certain amount for growth. Sargassum needs P as ion PO_4^- and this element is known as macro nutrient (Lobban and Harrison, 1997). The content of phosphate that is higher than tolerance limits will cause stunted growth. The current velocity is component in Panjang Island and Ujung Piring. Depth is the component in Bandengan station. Current and depth are hydrodynamic factors that positively and negatively influence Sargassum life.

Depth of water is related to current velocity. In shallow waters, current velocity tends to be bigger than the current velocity in the deeper water. Current can cause tissue damage in Sargassum because of erosion or stirred muddy base substrate resulted in turbidity and inhibit photosynthesis process.

The component in Teluk Awur is pH and in Bondo is DO. pH and Do are directive factors that have roles to direct biochemistry reaction in water ecosystem. Concentration of pH influences fertility and life of microorganisms. Acid water tends to cause death. The pH value in the water is influenced by CO_2 concentration which is acid substance. Seaweed and water vegetation absorb CO_2 from the water during photosynthesis is going on so that pH tends to increase in the noon and decrease in the night. The existence of O_2 is important for chemical-biological process in water and O_2 is needed in oxidation process for many kinds of water chemical substances (Dahuri et al., 1996; Lobban and Harrison, 1997).

Result of antibacterial activity shows that *S. aureus* is sensitive (6.28 ± 0.04 mm) to extract *S. echinocarpum* in ethyl acetate solvent from Bandengan. Bacteria *E. coli* is sensitive (6.96 ± 0.19 mm) to extract *S. echinocarpum* in ethyl acetate solvent from Teluk Awur. Bacteria *S. epidermidis* is sensitive (12.65 ± 0.23 mm) towards extract *S. binderi* in ethyl acetate solvent from Ujung Piring.

Result of cluster analysis shows that there is a similarity (99.73%) characteristic of water quality in Panjang Island and Bandengan station. It is probably because two stations are closely located compared to the other stations and similarity to Teluk Awur is 99.62%, Ujung Piring 99.60% and Bondo 99.52%. Water cluster analysis shows that Panjang Island is like central cluster, if the stations are arranged based on the closest distance to Panjang Island station results in *i.e.*: Bandengan, Teluk Awur, Ujung Piring, and Bondo. The distance has the same value with result of water quality cluster. Beside that terrestrial activities, beach topography and distance of one station to another will influence the differences of station's water quality. Result of cluster analysis of transparent zone diameter extract activity in each station shows that there is a similarity (99.99%) in Panjang Island station and Bandengan, while in Teluk Awur, Ujung Piring, and Bondo were 99.93%, 99.75% and 99.61%, respectively. The similarities of water quality cluster pattern and cluster of transparent zone diameter extract activity shows that there is a linkage between water quality to active compounds produced by Sargassum (Figure 2 and 3).

Table 2. Bioactivity Test of Sargassum Extract as MDR Anti-Bacteria

Station	Code Species	Solvent	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Staphylococcus epidermidis</i>	
Teluk Awur	<i>S duplicatum</i>	n-hexane	-	-	4.34 ± 1.10	
		Ethyl acetate	3.62 ± 0.05	4.22 ± 1.23	6.81 ± 1.46	
		Methanol	4.35 ± 0.32	5.87 ± 0.98	3.84 ± 1.57	
	<i>S polycystum</i>	n-hexane	-	-	3.54 ± 0.72	
		Ethyl acetate	5.88 ± 0.25	5.52 ± 0.81	6.44 ± 0.76	
		Methanol	1.94 ± 0.33	2.71 ± 0.29	2.06 ± 0.91	
	<i>S echinocarpum</i>	n-hexane	-	-	4.49 ± 0.58	
		Ethyl acetate	4.92 ± 0.18	6.96 ± 0.19	-	
		Methanol	2.01 ± 0.29	-	1.70 ± 0.77	
Panjang Island	<i>S cinereum</i>	n-hexane	-	-	-	
		Ethyl acetate	3.65 ± 1.22	6.63 ± 1.02	4.98 ± 0.52	
		Methanol	-	2.45 ± 0.12	2.03 ± 0.27	
	<i>S polycystum</i>	n-hexane	-	-	-	
		Ethyl acetate	4.13 ± 0.24	5.32 ± 0.69	6.22 ± 0.97	
		Methanol	2.27 ± 0.41	3.03 ± 0.28	4.64 ± 0.96	
	<i>S crassifolium</i>	n-hexane	-	-	-	
		Ethyl acetate	5.30 ± 0.38	5.98 ± 0.47	4.17 ± 0.31	
		Methanol	3.25 ± 0.01	-	2.00 ± 0.29	
	<i>S duplicatum</i>	n-hexane	4.23 ± 0.20	-	4.52 ± 0.49	
		Ethyl acetate	5.96 ± 0.35	4.98 ± 0.44	6.52 ± 1.67	
		Methanol	3.25 ± 0.01	-	2.00 ± 0.29	
	Bandengan	<i>S plagyophyllum</i>	n-hexane	-	-	-
			Ethyl acetate	4.62 ± 0.15	5.02 ± 1.20	5.23 ± 1.21
			Methanol	2.62 ± 0.11	-	2.24 ± 0.84
<i>S echinocarpum</i>		n-hexane	-	-	-	
		Ethyl acetate	6.28 ± 0.04	4.03 ± 0.88	4.90 ± 0.70	
		Methanol	1.91 ± 0.20	2.82 ± 1.08	3.06 ± 0.71	
<i>S cinereum</i>		n-hexane	-	-	-	
		Ethyl acetate	5.82 ± 0.95	5.66 ± 0.98	5.35 ± 1.07	
		Methanol	-	-	3.88 ± 0.34	
Ujung piring	<i>S binder</i>	n-hexane	-	-	-	
		Ethyl acetate	-	-	12.65 ± 0.23	
		Methanol	-	-	11.33 ± 0.44	
	<i>S polycystum</i>	n-hexane	-	-	-	
		Ethyl acetate	-	-	4.57 ± 0.16	
		Methanol	-	-	4.35 ± 0.42	
	<i>S duplicatum</i>	n-hexane	-	-	-	
		Ethyl acetate	-	-	-	
		Methanol	-	-	-	
Bondo	<i>S binder</i>	n-hexane	-	-	-	
		Ethyl acetate	-	-	5.16 ± 0.13	
		Methanol	-	-	-	

Information: data are the average ± standard deviation of bactericidal activity zone (mm)

Table 2. Result of Cluster Analysis Diameter Transparent Zone which has Bactericidal Activity and Large Spectrum

Cluster	Station	Variable Species	Solvent	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Staphylococcus epidermidis</i>
Cluster 1	Teluk Awur	<i>S. duplicatum</i>	Ethyl acetate	3.62 ± 0.05	4.22 ± 1.23	6.81 ± 1.46
	Panjang Island	<i>S. crassifolium</i>	Ethyl acetate	5.30 ± 0.38	5.98 ± 0.47	4.17 ± 0.31
	Bandengan	<i>S. cinereum</i>	Ethyl acetate	5.82 ± 0.95	5.66 ± 0.98	5.35 ± 1.07
Cluster 2	Panjang Island	<i>S. polycystum</i>	Ethyl acetate	4.13 ± 0.24	5.32 ± 0.69	6.22 ± 0.97
	Panjang Island	<i>S. duplicatum</i>	Ethyl acetate	5.96 ± 0.35	4.98 ± 0.44	6.52 ± 1.67
	Bandengan	<i>S plagyophyllum</i>	Ethyl acetate	4.62 ± 0.15	5.02 ± 1.20	5.23 ± 1.21
Cluster 3	Panjang Island	<i>S. cinereum</i>	Ethyl acetate	3.65 ± 1.22	6.63 ± 1.02	4.98 ± 0.52

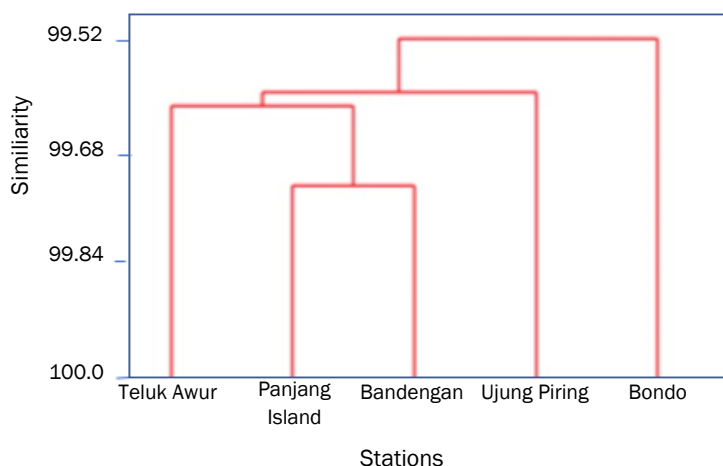


Figure 2. Cluster Analysis of Water Quality of All Stations

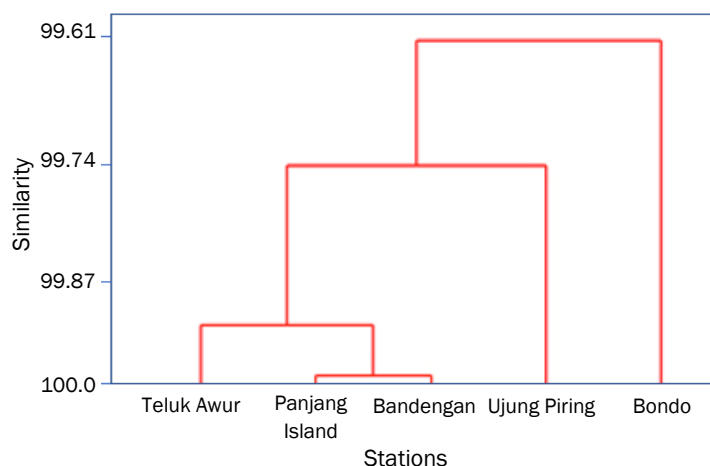


Figure 3. Cluster Analysis of Diameter Transparent Zone of Sargassum Extract Activity of All Stations

Extract with bactericidal activity in large spectrum then being classified into 3 classes (Similarity class 1, 2 and 3 are 72.33%; 76.80%; dan 58.50%). Class 1 has the best activity than class 2 and 3. Class 1 consists of *S. duplicatum* from Teluk Awur with the average bioactivity 5.37 mm; *S. crassifolium* from Panjang Island with the average of bioactivity 5.35 mm and *S. cinereum* from Bandengan with the average of bioactivity 5.32 mm (Table 1).

If species abundance in each station is linked, *S. duplicatum* from Teluk Awur has highest abundance, which is 504 ind.200m⁻², *S. crassifolium* from Panjang Island has highest abundance of 337 ind.200 m⁻², and *S. cinereum* from Bandengan has highest abundance of 329 ind.200 m⁻². Sargassum extract with the best activity has a linkage with abundance and linier positive relationship. Thus, Sargassum has the best activity and the best abundance (dominant) in its

ecosystem. It is assumed that active compounds which are isolated have potency as antimicrobial that has been functioned by Sargassum and ecologically is for natural survival (Table 2).

Conclusion

The abundant and antimicrobial bioactive compound has linkages and assumed as the primary metabolite product of Sargassum. Water quality parameters affects the abundance and biochemical content of Sargassum.

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