Antibacterial Activity of *Sargassum polycystum* and *Ulva reticulata* Methanol Extract Against Marine Fouling Bacteria

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ABSTRACT- Marine biofouling causes a lot of damage to the shipbuilding and aquaculture industry because of the increasing maintenance costs. The purposes of this research are to measure the effectiveness of seaweed to inhibit bacterial biofilms and determine their inhibitory concentrations. In this study, we used *Sargassum polycystum* and *Ulva reticulata* from Punaga coast, Takalar South Sulawesi, extracted with methanol to determine the potential of the extract as antifouling. There are 50 gr of *Sargassum polycystum* and *Ulva reticula*ta was extracted with 300 ml of each solvent (1:6 w/v) for three times maceration The highest antibacterial activity assay using agar diffusion method was indicated by *Sargassum polycystum* extract with inhibition zone range from 13.35 to 15.80 mm. Antifouling activities from brown algae make them very promising as candidates for eco-friendly antifouling.

Keyword: Seaweed. Methanol. Bacteria. Antifouling. Marine

I. INTRODUCTION

Solid substrate surface, when submerged in seawater, will experience changes formed by the results of attaching marine organisms (organism fouling), especially from microbes, diatoms, barnacles, tunicates, bryozoans and spores from seaweed. The implications of biofouling vary as the fisheries sector and shipping industry must face the consequences of biofouling (Plouguerné *et al*., 2010)). On ships, biofouling will increase ship weight, increase hydrodynamic drag and reduce ship maneuverability resulting in increased costs through increased use of labor, fuel, and docking time (Bazes *et al.*, 2009).

At first, controlling biofouling use chemicals in antifouling paint. The use of tributyltin (TBT) is the most successful way to eliminated biofouling. However, paint containing TBT harms non-target marine organisms. Efforts to prevent the attachment of fouling organisms to coastal buildings and ships are done by painting the building with paint containing heavy metals. Almost all paints used contain heavy metals with certain concentrations. The use of heavy metals as raw material for paint as one of the sources of heavy metal pollution which eventually accumulates in the sea. Pollution and decreasing environmental quality and environmental degradation are some of the impacts caused by the use of heavy metals (Bazes *et al.*, 2009).

Park *et al.* (2012) evaluated the ecological risk that Gomphina veneriformis growth was significantly delayed, the gonad index decreased and the changed sex balance (imposex) and intersex gonad in females increased significantly. In Maceio Coast, Brazil, *Thais rustica* has the imposex syndrome that makes male animals become females because of an impaired endocrine system (Camillo, *et al*., 2004).

In Indonesia, several studies have also been carried out regarding the dangers of using paint mixtures which are harmful to the lives of non-target organisms. Mamonto *et al.*, (2017) conducted research in Bitung, North Sulawesi using *Thais aculeata*, *Monodonta labio*, and *Nerita exuvia*. As a result, imposex in the region is quite high at around 58.5% - 80.67%.

This impact apparently does not only occur in shellfish but also occurs in *Kappaphycus alvarezii* algae. Because of these impacts, the International Maritime Organization on January 1, 2008, banned the use of paint from TBT throughout the world.

The application of biotechnology to produce natural material products from marine organisms generally does not cause effects and naturally biodegradable. The alternative prevents the presence of attachment biota by utilizing the active ingredients derived from nature, especially the sea. Efforts to combat attachment biota using bioactive compounds. It is more efficient and environmentally friendly alternative (Sumarno, 2017). One of the best ways to replace antifouling paint from TBT with natural compounds from marine organisms that have the potential to be antifouling. Seaweed is one of the many organisms reported as potentially antifouling.

Previous studies have been conducted to see the potential of seaweed as an antifouling. Some types of seaweed used are *Sargassum duplicatu*m (Santi, *et al.*, 2014), *Delisea pulchr*a (Steinberg, *et al*., 1998), *Stypopodium zonale, Dictyota menstrualis* and *Laurencia obtusa* (Da Gama, *et al.*, 2002), and *Bifurcaria bifurcata* (Maréchal *et al.*, 2004) has been reported to show significant antifouling activity. This study focuses on preventing biofouling at the stage of primary biofilm formation by bacteria and evaluates it by looking at its effects at the next stage of fouling. This research aims to know the potential of *Sargassum polycystum* and *Ulva reticulata* extract as natural antifouling.

II. MATERIALS AND METHODS

Collection of Seaweed Samples

Samples of *Sargassum polycystum* and *Ulva reticulata* were collected from Punaga coast, Takalar, Indonesia. The algae were washed with cleaned sea water and put into plastic bags before kept in a cool box to prevent photolysis and thermal degradation during transportation.

Sample Preparation

The samples were washed with sea and fresh water and sterile aquadest. After the washing process is complete the sample is drained until it is completely drained by the method of drying at room temperature for 4-6 days. Dry seaweed samples are made simplicia powder for the extraction process. The extraction process is done using methanol as a solvent. The extraction procedure was carried out by weighing 50 g of *Sargassum polycystum* seaweed powder in an Erlenmeyer flask and added 200 mL of methanol for 24 hours at room temperature. The solution was passed through Whatman No. 1 filter paper. The samples were dried using a rotary evaporator at a temperature of 45-50^{0}C and low pressure (500-700 mmHg vacuum). Seaweed extract is prepared for use in the antimicrofouling test.

Isolation of Marine Fouling Bacteria

Isolation of biofilm bacteria was carried out using a modified method of Sabdono, *et al.*, (2007). A 15 x 10 cm wood is attached to the substrate of water at a depth of 50 cm below sea level at the lowest low tide. The panel is taken after 2 weeks of immersion and put into the cool box and brought to the laboratory for the isolation of biofilm bacteria. The panel is sprayed with sterile seawater, then swabbing the panel surface with a sterile cotton swab to Tryptic Soy Broth (TSB).

Antibacterial Activity Test

Antibacterial activity test carried out between *Sargassum polycystum* and *Ulva reticulata* crude extract on biofilm bacteria using standard disc diffusion. The agar diffusion method was used for assessment of antibacterial activity (Zainuddin, *et al.*, 2019). 1 ml bacteria was inoculated in 20 ml of Tryptic Soy Agar (TSA). The warm agar containing bacteria then poured into Petri disc (9 cm in diameter) to cool down in laminar air flow at room temperature.

The crude extract of each seaweed (*Sargassum polycystum* and *Ulva reticulata*) was added a 50 µL at 2 mg concentration into a sterile paper diss (6 mm in diameter) (Zainuddin *et al.*, 2019). The paper disc was evaporated until dry then the disc

placed on the agar surface with bacteria and incubated for 24 hours at 37 ^{0}C. The negative controls are solvent which used during extraction (methanol). As a comparison, 4% of antifouling paint is used as a positive control. The antibacterial activity of Sargassum polycystum and Ulva reticulata extracts against marine fouling bacteria were assessed by measuring the diameter of the inhibition zone (clear zone). All treatments replication two times and every time with three replicates.

III. RESULT AND DISCUSSION

Seaweed that has been taken from Punaga coast is cleaned and washed to remove organisms, mud or sand. After being washed, the seaweed is then weighed as wet weight, as shown in ***Figure 1.*** Seaweed is dried in a shaded place and protected from direct sunlight for 4-6 days and weighed as dry weight. The wet weight of Ulva reticulata is 3250 gram and Sargassum polycystum is 3750 gram. Each seaweed was weight 50 gram to solve in 300 mL of methanol. Percent of dry biomass is 27.73% for *Sargassum polycystum* and 25.85% for *Ulva reticulata*, and percent of rendemen for *Sargassum polycystum* is 2.21% and 2.05% for *Ulva reticulata*. The highest percent of the crude extract is shown by *Sargassum polycystum* with methanol extract (***Table 1***).

Figure 1. Fresh seaweed from Punaga coast, Takalar.
Ulva reticulata (left) *Sargassum polycystum* (right)

Methanol solvent was also carried out as a comparison with the previous method. The percentage of crude extract from this method is the most among the others 2.21% for *Sargassum polycystum* and 2.05% for *Ulva reticulata*. This is related to solvent polarity. The inhibitory test results showed that methanol extract with this method also showed good results.

Table 1. Wet Weight and Dry Weight of Algae Biomass and Percentage of Crude Extracts Obtained from 50 g of Dry Weight Biomass in 300 mL Organic Solvent

No.	Species	Class	Wet-weight (ww)(g)	Dry Weight (dw)(g)	Percent of dw/ww	Solvent	Crude Extract (mg)	Crude Extract (%)
1.	*Sargassum polycystum*	Phaeophyta	3750	1040	27.73	Methanol	1.10	2.21
2.	*Ulva reticulata*	Chlorophyta	3250	840	25.85	Methanol	1.03	2.05

Antibacterial activity test using the calipers is done three times. All treatments were a replication of two times and every time with three replicates. Visual observations can be seen in Figure 1. The average diameter of *Sargassum polycystum* extract is 14.50 mm and 14.13 mm while *Ulva reticulata* extract is 13.18 mm and 12.44 mm. This is very good if compared with positive controls of 15.35 mm and 15.02 mm. The negative control is negative control does not provide a clear zone, it means that the solvent (methanol) does not have an effect on the inhibition of extract. The difference in inhibition of the

growth of biofilm bacteria by extracts produced by the bioactive composition available in each extract is different. Accordingly, it causes the ability to extract as antifouling differently.

Figure 2. Result of antibacterial activity : (1) *Ulva reticulata*, (2) *Sargassum polycystum* (3) Negative control, (4) Positive control. High antibacterial activity was shown by *Sargassum polycystum* with methanol solvent.

The best seaweed extracts against marine fouling bacteria were shown by *Sargassum polycystum* extract. Which can inhibit the most biofilm bacteria with a range of inhibition zone diameters from 11.10 to 18.30 mm. This shows that methanol extract has a broad spectrum as antimicrofouling which can inhibit various types of biofilm bacteria **(*Figure 2)***. Methanol extract has the potential as an antifouling because it can inhibit various types of biofilm bacteria that are the cause of microfouling.

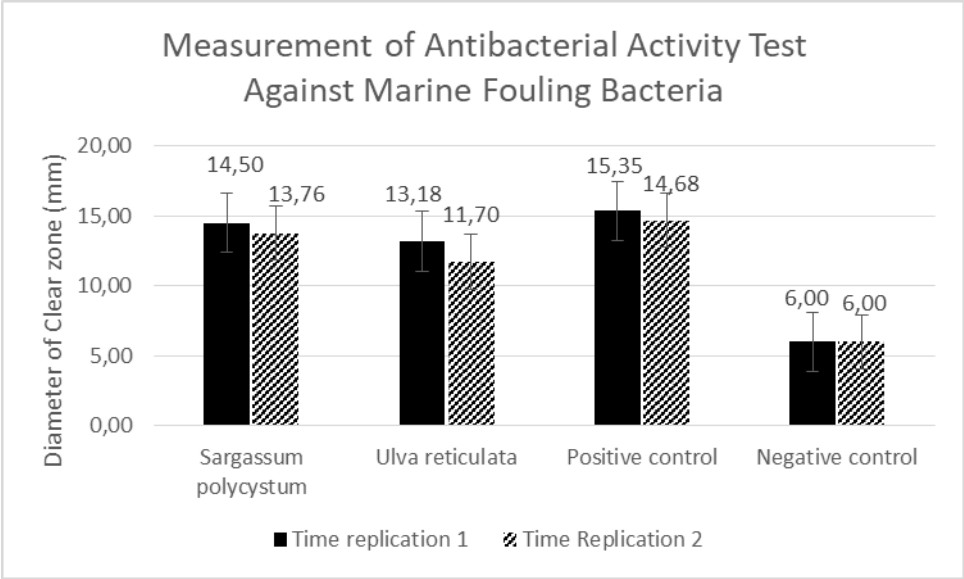

Figure 3. Mean of clear zone measurement in antibacterial activity test. Extract of seaweed with different solvent against marine fouling bacteria. A number of replicates are 6 per treatment.
*Inhibition zones include the disc diameter of 6 mm; the activity is categorized according to the diameter of the inhibition zone around the disc, ≥ 20 mm =highest, < 20–≥ 15 mm = high, < 15–≥ 10 mm = moderate, < 10 mm–> 6 mm = low, 6 mm = no activity (Elmi *et al.*, 2019)

The first step for controlling biofouling is by inhibiting the occurrence of the process of attaching larvae from invertebrate or spores from algae (microfouling). If at the initial stage the formation of biofilm bacteria (microfouling) can be inhibited it is thought that it can also inhibit the attachment of macrofouling so that biofouling can be controlled. S. duplicatum crude extract has the potential as an environmentally friendly antifouling because the control of biofouling comes from the secondary metabolites of marine organisms.

This secondary metabolism inhibits biofilm formation so that biofilm growth can be inhibited. The methanol extract of *Sargassum polycystum* contains alkaloid compounds, saponins, quinones, phenolics, steroids, and flavonoids. Whereas, S.

duplicatum ethyl acetate extract contains alkaloid compounds, saponins, steroids and flavonoids (Santi *et al.*, 2014). According to Da Gama *et al.* (2002), phenols and quinones are often found in the form of glycosides, which are present in cell vacuoles and are easily soluble in polar compounds (methanol). Alkaloids are found in extracts methanol. Alkaloids have nitrogen bases in their cyclic chains and contain a variety of substituents which vary as amine groups, amides, phenols, and methoxy so that alkaloids are semipolar. Alkaloids have antimicrobial activity against gram positive and negative bacteria (Putranti, 2013). This secondary metabolite is thought to interfere with biofilm formation so that the growth of biofilm bacteria.

IV. CONCLUSION

Seaweed extract is proven to be a candidate for natural antifouling which can inhibit marine fouling bacteria growth. The strong antifouling activity makes them promising candidates for new antifouling additives.

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