Antibacterial Activity of *Sargassum Polycystum* Against *Aeromonas hydrophila* and *Vibrio harveyi*

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Abstract

The use of synthetic antibacterials wich have been applied in cultivation activities can stimulate a negative impact to the environment and biota. Hence, it is important to find the antibacterial agent from natural source. *Sargassum polycystum* is one of the potential seaweed wich has not received attention and often considered as coast pollutant, even though this species contains high bioactive compounds. One of the potential use of *S. polycystum* is as natural antibacterial agent for *Aeromonas hydrophila* and *Vibrio harveyi*. The purpose of this study were to investigate the bioactive compounds contained in crude extract of *S. polycystum* qualitatively and their potential as antibacterial agent of *A. hydrophila* and *V. harveyi*. This research used experimental method with five treatments, three replications, for each bacteria test. The inhibition zone data was analysed with two way ANOVA, and the significant result was compared with Tukey HSD advanced test. The qualitative bioactive compound test showed that the *S. polycystum* extract contained tannins, flavonoids, and saponins. From the inhibition test revealed that *S. polycystum* extract has the potential as antibacterial for *A. hydrophila* and *V. harveyi* with weak inhibitory. The statistical analysis showed that at the 24-hour incubation time, there were significant difference between the treatments for the inhibition zone diameter of the *S. polycystum* extract, whereas at the 48-hour incubation time there were significant difference on the inhibition zone diameter in both the experimental treatments and the tested bacteria ($p \leq 0,05$).

Keywords : *Sargassum polycystum,* antibacterial*, Aeromonas hydrophila, Vibrio harveyi*

1. Introduction

Indonesia is the second largest seaweed producing country in the world after China (Ferdouse et al., 2018). In 2019, Indonesian seaweed production reached 9,9 million tons and is targeted to reach 10,99 million tons by 2020 (KKP, 2020). Seaweed commodities that grow in Indonesian waters include *Gracilaria*, *Gelidium*, *Euchema*, *Hypnea*, *Turbinaria* and *Sargassum* (Pambudi et al., 2010). One of the potential seaweed species from the genus *Sargassum* is *Sargassum polycystum*. The existence of this species in the community has not received special attention when compared to other species, even this species is considered to pollute the coast. The utilization of *S. polycystum* is still very low. This species is often neglected becaused it occurs abundantly in a certain season and interferes shipping activities. However, *S. polycystum* contains very useful bioactive compounds that might be useful for antibacterial and many other applications (Alamsyah et al., 2014).

Seaweed *S. polycystum* contains tannins, phenols, alginates, steroids / triterpenoids, saponins,

flavonoids, polyphenols, and alkaloids with a total phenol content of 713-1082 mgGAE / g (Manteu et al., 2018; Maulina, 2019; Riwanti & Izazih, 2019). The content of these bioactive compounds indicates that *S. polycystum* has the potential to be a natural antibacterial agent. Natural antibacterials are antibacterials that are obtained directly from the organism that produces these compounds by carrying

out the extraction process (Septiani et al., 2017). Extract of *S. polycystum* has the potential as a natural antibacterial against pathogenic bacteria, for example against *A. hydrophila* and *V. harveyi*.

Aeromonas hydrophila and *V. harveyi* are pathogens of aquatic organisms, either freshwater or seawater (Arifin et al., 2017). *A. hydrophila* is a gram-negative rod-shaped bacteria. These bacteria causes a bacterial disease called *Motile Aeromonas Septicemia* (MAS) or also known as hemorrhage septicemia (Kurniawan, 2016). In Southeast Asia, this disease first occurred in the West Java region in 1980 which caused the death of 82,2 tons of freshwater fish in a month. *Motile Aeromonas Septicemia* (MAS) also occurred in the Banyumas region, which attacked 72.000 freshwater fish in 2003 and caused the death of parent carp with a mortality rate of 90% in 2017 (Khumaidi & Hidayat, 2018; Mulia, 2007; Prayogi, 2016). *V. harveyi* (luminous bacteria) which can cause vibriosis disease (Novriadi et al., 2014). Vibriosis in white shrimp *L. vannamei* is a disease that causes the most economic losses (Li & Xiang, 2013). According to Flegel & Sritunyalucksana (2011), the economic loss of vibriosis in shrimp farming reaches 1 billion USD annually in the world.

So far, the handling of pathogenic bacteria such as *A. hydrophila* and *V. harveyi* uses synthetic antibacterials such as oxytetracyclin, prefuran, and ampicillin (Setyaningsih et al., 2006). The use of synthetic antibacterials can cause several disadvantages, including bacterial resistance to antibacterials and causing residues on the bodies of aquatic organisms. The accumulation of residues in aquatic organisms has the potential to damage the health of humans and animals that consume them. Apart from that, the waste also affects the condition of water quality and the surrounding environment. Hence, the developed countries prohibit the use of synthetic antibacterials and fishery products that contain synthetic antibacterial residues (Sukenda et al., 2006). An alternative of antibacterials source from *S. polycystum* is needed to overcome the impact caused by the use of synthetic antibacterials because the use of natural antibacterials is more environmentally friendly and can suppress the growth of pathogenic bacteria without causing resistance. Therefore, this study was conducted to determine the potential of *S. polycystumagent* as an antibacterial for *A. hydrophila* and *V. harveyi*.

2. Materials and Methods

Material

The tools used in this study include analytical scales, blender, waterbath, shaker incubator, rotary evaporator, autoclave, petri dish, paper disk 6 mm, and incubator. The materials used in this study included samples of seaweed *S. polycystum,* bacterial isolates of *A. hydrophila* and *V. harveyi,* TSA media, ampicillin, methanol PA, aquades, $FeCl_3$ 1%, Mg powder, HCL, KOH, Alcohol 70 %, and methylated spirits.

Method of Extraction S. polycystum

Secondary metabolite extraction was carried out by maceration extraction. Maceration was carried out using a polar solvent, namely the methanol PA solution. A total of 100 g of dry sample *S. polycystum* was crushed and put into erlenmeyer and then 200 mL of methanol PA solution was added. The sample was then homogenized using an shaker incubator with a speed of 125 rpm for 2x24 hours. Every 24 hours the sample was filtered using filter paper Whatman No. 41 and the residue was added with the solvent then homogenized again. The maceration results were put together and then

evaporated using a rotary evaporator at a temperature of ± 60 ^{0}C until the solvent is evaporated. The sample was again concentrated using a water bath with a temperature of ± 60 ^{0}C and stirred periodically. The crude extract obtained was then weighed and the yield value was calculated. The yield was obtained from the comparison of the extract weight and the initial sample weight which is expressed in units of percent (%). The formula for calculating the yield value refers to AOAC (1995) in Podungge et al. (2018), mathematically written as follows:

$$\% \text{ yield} = \frac{\text{Extract Weight}}{\text{Initial Sample Weight}} \times 100\%$$

Phytochemical Analysis

a. Tannin Test

Testing for tannin compounds was carried out by mixing 0.1 g of extract *S. polycystum* into 10 mL of hot water and boiling for 5 minutes then filtering. The filtrate was added with 10 mL of $FeCl_3$ 1%. A positive test for the presence of tannins was indicated by the appearance of a blackish green color.

b. Flavonoid Test

Flavonoid test was carried out using 2 mL of sample (0,05 % w / v) added to 2 mL of hot water, boiling for 5 minutes, then filtering. The filtrate was added with 0,05 mg of Mg powder and 1 mL of HCl, then homogenized. Flavonoid compounds were indicated by the formation of red or orange to yellow colors.

c. Saponin Test

The saponin test was carried out using 2 mL of sample (0,05 % w / v) dissolved in distilled water in a test tube, added with 10 drops of KOH and heated in a water heater ± 50 ^{0}C for 5 minutes, then shaked for 15 minutes. If foam was formed and remained stable for 15 minutes, it indicated the presence of saponin compounds.

Antibacterial Activity Test Based on Inhibition Zones

Antibacterial activity of extract *S. polycystum* can be determined by testing using the Kirby-Bauer agar diffusion method in (Hudzicki, 2009) with modification. The stages include preparation of tools and materials, sterilization of tools and materials using an autoclave at a temperature of ± 121 ^{0}C for 15 minutes, rejuvenating the test bacteria, and testing the antibacterial activity. The rejuvenation of the tested bacteria was started from planting the bacteria on the selective media, namely *V. harveyi* on TCBS media and *A. hydrophila* on GSP media then incubated at ± 28 ^{0}C for 24 hours. The bacteria that grew respectively were streaked on TSA media using a loop needle, then incubated at a temperature of ± 28 ^{0}C for 24 hours. The final step in bacterial rejuvenation was planting the bacteria from the TSA media into the TSB media and then inserting a shaker incubator at a speed of 200 rpm for 24 hours. The bacteria test consisting of *A. hydrophila* and *V. harveyi* were the independent variables in this study.

The antibacterial activity test based on the inhibition zone diameter started from planting the test bacteria on TSA media using the method spreadplate with the hokey stick spreader. Then the sterile disc paper measuring 6 mm was given the treatment as in Table 1. The treatment was done by placing sterile disc paper into a sterile dish, then dropping 10 µL of the treatment as in Table 1 using a micropipette with aseptic conditions until the solution was absorbed into the disc paper. Furthermore, the discs that have been treated are placed on the TSA media using tweezers.

Table 1. Treatment antibacterial testing

Materials	Solvents	Concentration (ppm)	Description
Extracts of *S. polycystum*	Aquades	50	Sample 1
Extracts of *S. polycystum*	Aquades	200	Sample 2
Extracts of *S. polycystum*	Aquades	800	Sample 3
Ampicillin	Aquades	1000	Control +
-	Aquades	-	Control -

Treatment of antibacterial testing in Table 1 was the independent variable in this study. Each treatment was given to both tested bacteria and each treatment was repeated 3 times. After being given the treatment, the samples were incubated at a temperature of ± 28 ^{0}C for 1x24 hours, with observations at the incubation time of 24 hours and 48 hours. The diameter of the clear zone formed was measured using the measured software was Image J. The diameter of the inhibition zone formed was the dependent variable in this study. So, in this study there are two independent variables, namely the bacteria test and antibacterial test treatment, and there was one dependent variable, namely the diameter of inhibition zone.

The diameter of the inhibition zone was determined by measuring the diameter of the clear zone formed. The method for measuring the diameter of the inhibition zone can be seen in Figure 1.

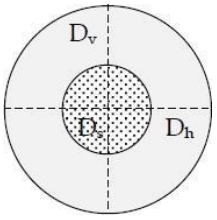

Figure 1. Diameter of the inhibition zone

The formula for calculating the average value of the inhibition zone diameter was as follows:

$$\text{Inhibition Zone Diameter} = \frac{Dv + Dh}{2} - Ds$$

Description:

D_v : Vertical diameter

D_h : Horizontal diameter

D_s : Diameter of paper disk

: Inhibition zone diameter

: Paper disk (Pormes et al., 2016).

The ability of the inhibition zone can be determined by referring to the category of Hutasoit et al. (2013) can be seen in Table 2.

Table 2. Category ability inhibition zone

Inhibition Zone Diameter	Power Inhibition of
≤ 5 mm	Weak
6-10 mm	Medium
11-20 mm	Strong
> 20 mm	Very strong

Data Analysis

Data obtained in the form of qualitative and quantitative data. Qualitative data, namely the results of phytochemical tests, were analyzed descriptively using tables. Quantitative data in the form of yield *S. polycystum* with methanol PA solvent were analyzed descriptively, while the inhibition zone

diameter was measured using software Image J. The inhibition zone diameter data were statistically analyzed and displayed in graphic form to make it easier to understand the data. Statistical analysis begins with the normality and homogeneity test. The data obtained was not normally distributed and homogeneous so that the data is transformed with the Yeo-Johnson transformation using the software Minitab 2017. This transformation can be used to normalize data and is suitable for zero or negative data (Yeo & Johnson, 2000). After the data were normally distributed and homogeneous, a Two Way ANOVA test was carried out using the software Minitab 2017. If the results were significant, then continued with the Tukey Test using the software SPSS 2017.

3. Results and Discussion

The sample used in this study was seaweed type *Sargassum polycystum* from Pantai Sayang Heulang, Garut, West Java which can be seen in Figure 2. *S. polycystum* is a type of brown seaweed that is widely found in Indonesian waters. *S. polycystum* is part of the genus *Sargassum* which is the largest genus of the family Sargassaceae (Noiraksar & Ajisaka, 2008). The general characteristics of *S. polycystum* are the shape of the thallus is generally cylindrical or flat, the branches are thick like trees on land, the leaf shape is seven broad, oval, or sword-like, has air bubbles, was generally solitary, and the color of the thallus is generally brown (Hidayat et al., 2018).

Figure 2. *Sargassum polycystum* from Pantai Sayang Heulang, Garut, West Java (personal document)

The results of this study consist of qualitative data and quantitative data. Qualitative data is the test results of the phytochemical compounds of extract *S. polycystum*, while quantitative data is the yield of extract *S. polycystum* and the diameter of the zone of inhibition against bacteria *A. hydrophila* and *V. harveyi* with an incubation time of 24 hours and 48 hours.

Yield of S. polycystum Extract

The maceration extraction of 100 grams of the sample *S. polycystum* using methanol PA solvent resulted in a crude extract of 2,36 grams with a yield of 2,36%. The resulting yield is lower than previous research which states that the yield of methanol extract *S. polycystum* ranges from 2,79 – 6,42% (Minarti et al., 2019; Savitri et al., 2017; Widowati et al., 2018). The factors that influence the high and low yield of seaweed are : seaweed species, harvest season, geographic location, extraction method, extraction time, extraction temperature, and type of solvent used for extraction (Junaidi, 2013; Kurnialahi et al., 2020; Pasanda & Azis, 2018).

Qualitative Test of Extract S. polycystum

Qualitative test conducted in this study was a phytochemical test which included: tannin, flavonoid, and saponin tests. The results of the phytochemical compound test can be seen in Table 3.

Table 3. Phytochemical test results of extracts *S. polycystum*

Phytochemical compounds	Positive	Test results
Tannin	Formed blackish green color	+++ 
Flavonoids	formed yellow to orange colors	+ 
Saponins	Formed stable foam	+++ 

Remarks : + : positive; - : negative. The more "+" sign shows more convincing results (Sibero et al., 2020).

It can be observed in Table 3 that extract *S. polycystum* has a high tannin content, low flavonoid content, and high saponin content. This is in accordance with the results of previous research which states that extract *S. polycystum* contains tannins, flavonoids, and saponins (Barodah et al., 2017; Manteu et al., 2018; Riwanti & Izazih, 2019). The low flavonoid content can be caused because flavonoids are unstable compounds against changes in the effects of oxidation, light, and chemical changes (Chikmawati et al., 2013). According to Pangestuti et al. (2017), the high and low content of phytochemical compounds can be influenced by the type of polarity of the solvent used for extraction. The high saponin content is due to the fact that saponins and methanol have the same polarity so that saponins dissolve easily in methanol solvents (Pangestuti et al., 2017).

The content of tannin, flavonoid, and saponin in extract *S. polycystum* is almost the same as some other seaweeds. *Galaxaura rugosa* contains flavonoids and saponins, but doesn't contain tannins (Bhernama et al., 2021). *Sargassum wightii* contains tannins and flavonoids but doesn't contain saponins (Balachandran et al., 2016). Based on Ramasubbu et al. (2012), *Codium adharens* and *Sargassum wightii* contains flavonoids and saponins, but doesn't contain tannins; *Acanthophora spicifera* contains flavonoids, but doesn't contain tannins and saponins. Mehdinezhad et al. (2016), stated that the most abundant compounds in *Sargassum angustifolium* were tannins and saponins, followed by flavonoids. Whereas, the most abundant compounds in *Sargassum oligocystum* and *Sargassum boveanum* were tannins, followed by flavonoids and saponins.

The content of tannin, flavonoid, and saponin compounds in extract *S. polycystum* has the potential to be antibacterial against bacteria *A. hydrophila* and *V. harveyi* with different mechanisms

between compounds. Tannins have the ability to bind to bacterial cell walls, inhibit bacterial growth, and protease activity. Flavonoids inhibit bacterial growth by damaging cell membranes and inhibiting the macromolecular synthesis of bacterial cells. Saponins inhibit bacterial growth by reducing the efficiency of glucose utilization, influencing growth and proliferation, reducing enzymatic activity, suppressing protein synthesis, then causing cell death (Mawan et al., 2018).

Potential of extract *S. polycystum* as antibacterial based on its inhibition zone diameter against Bacteria *A. hydrophila* and *V. harveyi*

The results of the antibacterial test of extract *S. polycystum* based on the diameter of the inhibition zone against *A. hydrophila* and *V. harveyi* can be seen in Figure 3. The clear zone around paper disc is an inhibition zone that shows the sensitivity of the tested bacteria to the given treatment.

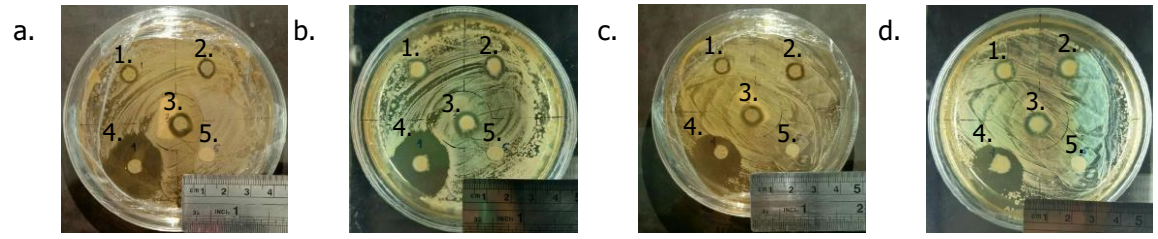

Figure 3. Antibacterial test results of extract *S. polycystum*. (a) bacteria *A. hydrophila* at 24 hours incubation, (b) bacteria *A. hydrophila* at 48 hours incubation time, (c) bacteria *V. harveyi* at 24 hours incubation time, (d) bacteria *V. harveyi* at 48 incubation time hour. (1) extract *S. polycystum* 50 ppm, (2) extract *S. polycystum* 200 ppm, (3) extract *S. polycystum* 800 ppm, (4) Control + (*Ampicillin*), (5) Control - (Aquades) (personal document)

The results of measurements of the inhibition zone diameter of extract *S. polycystum* against bacteria *A. hydrophila* and *V. harveyi* 24 and 48 hours of observation can be seen in Tables 4 and 5 and in graphical form in Figure 4.

Table 4. Antibacterial test results based on the inhibition zone diameter in incubation time 24 hours

Treatment (ppm)	Bacteria Test	
	***A. hydrophila* (mm)**	***V. harveyi* (mm)**
50	$2,16 \pm 0,22^{c}$	$2,23 \pm 0,51^{c}$
200	$3,59 \pm 0,34^{b}$	$2,90 \pm 0,37^{b}$
800	$5,00 \pm 0,10^{b}$	$3,92 \pm 0,52^{b}$
Ampicillin (Control +)	$23,80 \pm 0,73^{a}$	$16,24 \pm 0,41^{a}$
Aquades (Control -)	$0,29 \pm 0,06^{d}$	$0,43 \pm 0,30^{d}$

Description: Mean values followed by different letters indicate a significant difference (*Tukey test*; p≤0.05).

Table 5.Antibacterial test results based on the inhibition zone diameter at 48 hours of incubation.

Treatment (ppm)	test bacteria	
	***A. hydrophila* (mm)**	***V. harveyi* (mm)**
50	$3,95 \pm 0,38^{C, \alpha}$	$3,15 \pm 0,70^{C, \beta}$
200	$4,89 \pm 0,56^{BC, \alpha}$	$4,06 \pm 0,13^{BC, \beta}$
800	$5,92 \pm 0,04^{B, \alpha}$	$5,19 \pm 0,79^{B, \beta}$
Ampicillin (Control +)	$21,28 \pm 0,88^{A, \alpha}$	$16,38 \pm 0,21^{A, \beta}$
Aquades (Control -)	$0,68 \pm 0,33^{D, \alpha}$	$0,41 \pm 0,31^{D, \beta}$

Description: Mean value followed by different letters (treatment) and symbols (test bacteria) in the same row and column shows a significant difference (*Tukey test*; p≤0.05).

Table 4 shows that the treatment given affects the diameter of the inhibition zone of the tested bacteria ($p \leq 0.05$). The highest inhibition zone diameter at 24 hours observation was found in antibiotic treatment Ampicillin (control +) of 23,80 ± 0,73 mm against *A. hydrophila* and 16,24 ± 0,41 mm for bacteria *V. harveyi*, while the lowest inhibition zone diameter was found in distilled water treatment (control -) of 0,29 ± 0,06 mm for bacteria *A. hydrophila* and 0,43 ± 0,30 mm for bacteria *V. harveyi*. All concentrations of extract *S. polycystum* resulted in inhibition zone diameter with the category of weak inhibition against bacteria *A. hydrophila* and *V. harveyi*. A concentration of 50 ppm resulted in a diameter of inhibition zone against bacteria *A. hydrophila* and *V. harveyi* higher than aquades treatment (control -) respectively, namely 2,16 ± 0,22 mm and 2,23 ± 0,51 mm. The inhibition zone diameter value was lower than the extract treatment at a concentration of 200 ppm and 800 ppm in the two groups of bacteria, respectively, in the range of 2,90 ± 0,37 mm to 5,00 ± 0,10 mm. The inhibitory power formed by administering the extract of *S. polycystum* against *A. hydrophila* and *V. harveyi* is in the weak inhibition category with a diameter range of the zone of inhibition against these bacteria of 2,16 – 5,00 mm.

The diameter of the inhibition zone against bacteria *A. hydrophila* on 24 hours observation showed that antibiotic treatment *Ampicillin* (control +) resulted in sequential inhibition zone diameters of 11,02 x; 6,63 x; 4,76 x stronger than the extract of *S. polycystum* 50 ppm, 200 ppm, 800 ppm. However, antibiotic treatment *Ampicillin* (control +) resulted in an inhibition zone diameter of 82,07 x stronger when compared to aquades treatment (control -). The diameter of the inhibition zone against bacteria *V. harveyi* at 24 hours observation showed that antibiotic treatment *Ampicillin* (control +) resulted in sequential inhibition zone diameters of 7,28 x; 5,60 x; 4,14 x stronger than the extract of *S. polycystum* 50 ppm, 200 ppm, 800 ppm. However, antibiotic treatment *Ampicillin* (control +) resulted in a 37,77 x stronger inhibition zone diameter when compared to distilled water treatment (control -) (*Tukey test*, $p \leq 0.05$).

Table 5 shows that the treatment and test bacteria had an effect on the inhibition zone diameter ($p \leq 0.05$). Bacteria *V. harveyi* gave a higher response to the treatment given compared to bacteria *A. hydrophila* (*Tukey test*, $p \leq 0.05$). The highest inhibition zone diameter at 48 hours of observation was found in antibiotic treatment *Ampicillin* (control +) of 21,28 ± 0,88 mm against *A. hydrophila* and 16,38 ± 0,21 mm for bacteria *V. harveyi*, while the lowest inhibition zone diameter was found in distilled water treatment (control -) of 0,68 ± 0,33 mm for bacteria *A. hydrophila* and 0,41 ± 0,31 mm for bacteria *V. harveyi*. All concentrations of extract *S. polycystum* resulted in inhibition zone diameter with the category of weak inhibition against bacteria *A. hydrophila* and *V. harveyi*. The concentration of 50 ppm resulted in a diameter of inhibition zone against bacteria *A. hydrophila* and *V. harveyi* higher than aquades treatment (control -) respectively, namely 3,95 ± 0,38 mm and 3,15 ± 0,70 mm. The value of the inhibition zone diameter was lower than the extract treatment at a concentration of 200 ppm and 800 ppm in the two groups of bacteria respectively, namely in the range of 4,06 ± 0,13 mm to 5,92 ± 0,04 mm. The inhibitory power formed by administering the extract of *S. polycystum* against *A. hydrophila* and *V. harveyi* is in the weak inhibition category with a diameter range of the inhibition zone against these bacteria of 3,15 – 5,92 mm.

The diameter of the inhibition zone against bacteria *A. hydrophila* at 48 hours of observation showed that antibiotic treatment *Ampicillin* (control +) resulted in the inhibition zone diameter of 5,39 x; 4,35 x; 3,59 x stronger than the extract of *S. polycystum* 50 ppm, 200 ppm, 800 ppm. However, antibiotic treatment *Ampicillin* (control +) resulted in a 31,29 x stronger inhibition zone diameter when compared to aquades treatment (control -). The diameter of the inhibition zone against bacteria *V. harveyi* at 48 hours of observation showed that antibiotic treatment *Ampicillin* (control +) resulted in the inhibition zone diameter 5,20 x; 4,03 x; 3,16 x stronger than the extract of *S. polycystum* 50 ppm, 200 ppm, 800 ppm. However, antibiotic treatment *Ampicillin* (control +) resulted in a 39,95 x stronger inhibition zone diameter when compared to aquades (control -) treatment (*Tukey test*, $p \leq 0.05$).

The inhibition zone diameter of extract *S. polycystum* in Tables 4 and 5 is the same as the inhibition zone diameter range of previous studies which stated that the inhibition zone diameter of extract *S. polycystum* was 3,12 mm against bacteria *S. aureus*; 4,15 - 14 mm against *E. coli bacteria*; 5,65 mm against bacteria *V. harveyi*; 6.4 mm against bacteria *M. luteus*; and 14 mm against bacteria *B. cereus* (Panjaitan & Madayanti, 2018; Prasetya et al., 2020; Riyanto et al., 2013). This indicates that *S. polycystum is* included in the category of weak inhibition in several types of bacteria such as *A. hydrophila, V. harveyi, S. aureus,* and *E. coli*. Meanwhile, *M. luteus is* included in the medium inhibitory category and bacteria is *B. cereus* included in the strong inhibitory category (Hutasoit et al., 2013).

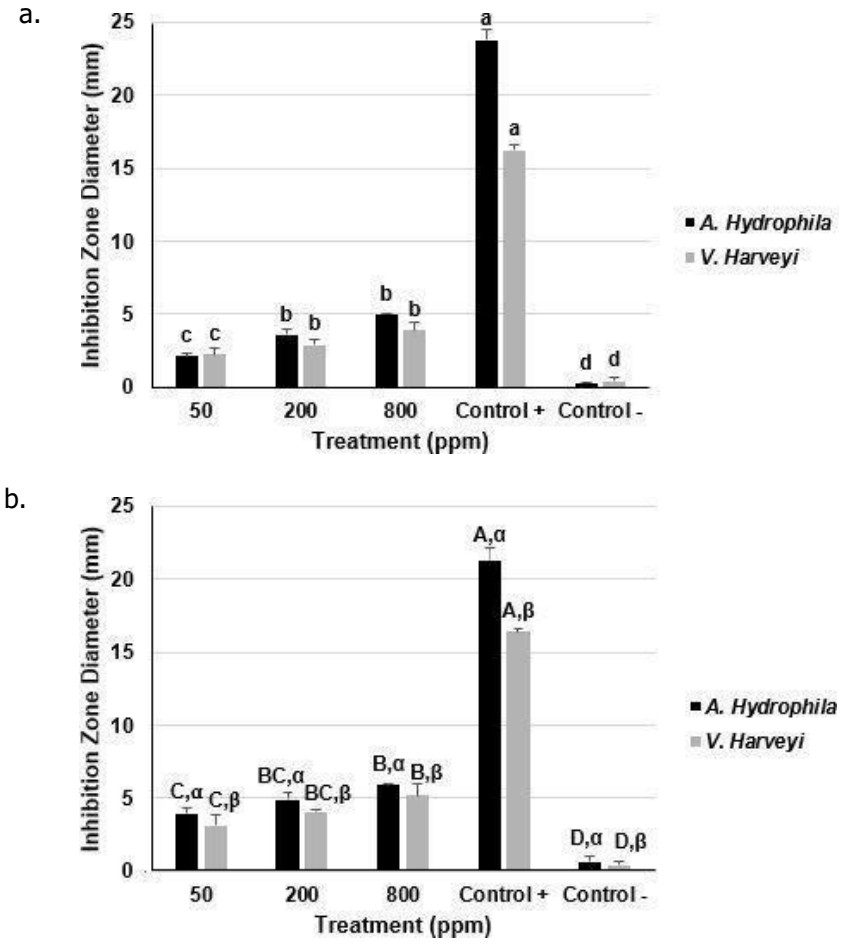

Figure 4. Mean ± SE (χ ± SE) inhibition zone diameter of extract *S.polycystum* against bacteria *A. hydrophila* and *V. harveyi*. (a) Incubation time of 24 hours, (b) incubation time of 48 hours. Different

letters (treatment) and symbols (test bacteria) on the bar graph in each treatment indicate a significant difference (*Tukey test*; p≤0.05)

The graphs in Figures 4 a and b show that the higher the concentration of extract *S. polycystum* used, the higher diameter of the inhibition zone formed, but the highest diameter of the inhibition zone was produced by the control (+), namely *Ampicillin* 1000 ppm. This is in accordance with the statement of Dwijoseputro (2003) *in* Alfan et al. (2017), the higher concentration of the solution, the greater inhibition of bacterial growth and affects the killing power of these bacteria.

The treatment of *S. polycystum extracts of* 50 ppm, 200 ppm, and 800 ppm resulted in an average diameter of the inhibition zone with the category of weak inhibition against bacteria *A. hydrophila* and *V. harveyi* (Hutasoit et al., 2013). Weak inhibition power can be caused because these bacteria are gram-negative bacteria. The cell wall in gram-negative bacteria consists of thin peptidoglycan and is mostly composed of a non-polar lipid membrane so that it is difficult to penetrate by the antibacterial compounds contained in the extract *S. polycystum* (Pangestuti et al., 2017). Control (+) produced an average inhibition zone diameter with very strong inhibition against bacteria *A. hydrophila* and strong inhibition against bacteria *V. harveyi* (Hutasoit et al., 2013). *Ampicillin* as a control (+) has the ability to inhibit microbial cell wall synthesis and inhibit the transeptidase enzyme and prevent cell biosynthesis (Akbar et al., 2016). *Ampicillin* is a broad-spectrum antibiotic that can inhibit the growth of gram-positive and gram-negative bacteria, as well as aerobic and anaerobic bacteria. Control (-) resulted in a weak inhibition zone diameter against bacteria *A. hydrophila* and *V. harveyi*. The choice of distilled water as a control (-) is because it is neutral and does not have the ability to inhibit bacterial growth (Putra et al., 2017).

The zone of inhibition that forms around the disc has various sizes. This can be caused by scratching the test bacteria that are not evenly distributed on the media so that the bacteria grow unevenly in every part of the media. Even though the test bacteria scratches evenly, the bacteria can also grow imperfectly so that no inhibition zone effect around the disc is formed. According to Prescott (2005) *in* Fitriah et al. (2017), the size of the inhibition zone is influenced by the level of sensitivity of the tested microorganisms, the diffusion rate of antibacterial compounds, and the concentration of antibacterial compounds. Factors that influence the speed of agar diffusion include : microorganism concentration, media composition, incubation temperature, and incubation time (Schlegel and Schmidt, 1994 *in* Siregar et al., 2012).

4. Conclusions

Extract *Sargassum polycystum* qualitatively contains tannins, flavonoids, and saponins. Extracts *S. polycystum* 50 ppm, 200 ppm, and 800 ppm produced an average of the inhibition zone diameter which is included weak inhibitory category against bacteria *A. hydrophila* and *V. harveyi*. Suggestions for further research is necessary to improve doses of *S. polycystum* extract to be used in research, as well as studies further in the quantitative determination of bioactive compounds in extracts *S. polycystum* and the potential as antibacterial against *A. hydrophila* and *V. harveyi*.

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