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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF BROWN ALGAE EXTRACT IN *SARGASSUM FILIPENDULLA* FROM SOUTHEAST MALUKU WATERS TOWARD THE *VIBRIO HARVEYII* AND *PSEUDOMONAS FLUORESCENCE* BACTERIA

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ABSTRACT

Vibrio harveyii and *Pseudomonas fluorescence* bacteria are pathogenic bacteria that are often a problem in aquaculture, so effective antibacterial compounds are needed to inhibit their growth. Brown algae of *Sargassumfilipendulla* have bioactive compounds that can function as antibacterial compounds. The purpose of this study was to determine the phytochemical content of *S. filipendulla* of algae extract from Southeast Maluku waters and to determine the antibacterial activity of *S. filipendulla* extract to *V. harveyii* and *P.fluorescence* bacteria. Phytochemical test parameters were carried out quantitatively; antibacterial activity was carried out using agar diffusion method (Kirby Bauer). Data from the inhibitory zone test results were analyzed using the ANOVA (Analysis of variance) test followed by the Least Significant Difference (LSD) test. The test results showed that the brown algae of *S. filipendulla* originating from the Southeast Maluku region, produced extracts in the form of green paste to blackish green with the highest rendemen value in ethanol extract. The results of phytochemical screening showed that the compounds contained in *S.filipendulla* extract were phenolic, tannin, flavonoid, saponin, triterpenoid, steroid and alkaloid compounds; with the dominant compounds in the three reactants were saponin compounds .The results of the research of inhibition zones showed that the concentration of 100% had the highest inhibition zone value and the lowest inhibition zone at a concentration of 75%. Ethanol extract has a high value to inhibit pathogenic bacterium *V.harveyi* and P. fluorescence and the lowest on ethyl acetate extract, whereas positive control is suspect against bacteria and negative control does not affect pathogenic bacteria. The 100% concentration of ethanol extract gave a significant effect on inhibiting pathogenic bacteria *V.harveyi* and P. fluorescence ($P < 0.05$). The 50% concentration gives a significant difference between ethanol and ethyl acetate extract, at the level of 0.05 ($P < 0.05$), while the n-hexane extract gives a non-significant result of ethanol and ethyl acetate extract. The average extract value to inhibit the highest pathogenic bacteria is in ethanol extract and the lowest is in n-hexane extract. Of the three extracts used, ethanol extract is an extract that is good for inhibiting pathogenic bacteria and has bacteriostatic properties.

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INTRODUCTION

Microalgae are fishery commodity that have high economic value and is a source of non-oil and gas income. The largest group of macroalgae can be classified into red algae (rhodophyta), brown algae (phaeophyta), and green algae (chlorophyta), which contain sources of nutrients and chemical composition. Red algae and brown algae are widely used in the food and medicine industry.In general, microalgae is widely used as a raw material for the food, cosmetics, pharmaceutical industries and others. Currently marine algae have been used as raw material for agar-agar, carrageenan and alginate (Anandhan, 2011; Alghazeer *et al.,* 2013; Harborne, 2003) industries. macroalgae is one of the important sources of producing bioactive compounds that have various biological activities including antibacterial, antifungal, antiviral, antitumor, antioxidant and anti-inflammatory. Many bioactive marine algae compounds with antibacterial activity have been isolated.Some of the compounds contained therein are sterols, terpenoids, polysaccharides, peptides, proteins, vitamins, acrylic acid, terpenes, chlorophyllides, phenols, heterocyclic compounds, halogenated ketones, alkanes and cyclic polysulphides (Anandhan, 2011). So that marine algae in both wet and dry forms are consumed by many people, especially those living in coastal areas. This is because biologically algae include chlorophyll plants which consist of one or many cells

and are in the form of colonies and contain organic ingredients such as polysaccharides, hormones, proteins, vitamins, essential minerals for the body. *Sargassum sp* is one type of seaweed that is found in Indonesian waters but has not been widely used when compared to commercial seaweed species *Eucheuma* and *Gracilaria.* Some European countries say that *sargassum sp* is an invasive species that develops rapidly so it competes with native species and can change community composition and ecosystem dynamics (Septiana, 2012). *Sargassum sp* can be used as a food ingredient, fuels, cosmetics (moisturizing crem), medicines, pigments, and additional food ingredients (Rajasulochana, 2009). *Sargassum* is a genus of brown algae found in many subtidal and intertidal regions, there are 150 species in the tropics. In fact there is still little research on the antibacterial activity of algae in Indonesia, especially *Sargassum.* Based on phytochemical tests from several studies conducted by *Sargassum sp* produces secondary compounds including fluoratanine, flavonoids, steroids, sterols, alkoloid, saponins and tannins (Alamsyah *et al.,* 2014).The research conducted by (Samee, 2009) about physicochemical extract of *S. duplicatum* obtained the quantitative results of the extract containing almost the same amount of flavonoids, saponins, tannins and terpenoids. *Sargassumsp* produces several secondary compounds such as fluoratanin (has antibacterial properties), flavonoids, steroids and sterols (Brooks *et al.,* 2007) where these compounds are known to be antibacterial.

Sargassum is active against vibrio bacteria using both water extract and methanol seen from a wider inhibition zone than other types. The formation of inhibitory zones is caused by the presence of secondary metabolites which are antibacterial (Guedes *et al.,* 2012; Rajasulochana *et al.,* 2009). Several studies have reported that the benefits of *Sargassumsp* in the field of pharmacology are antibacterial. Therefore the approach used in applying *Sargassumfilipendulla* from Southeast Maluku waters as a natural medicine is to study the inhibition of *Sargassum*extract to pathogenic bacteria. The purpose of this study was to determine the phytochemical content of brown algae extract of *S. filipendulla* and determine the antibacterial activity of *S. filipendulla* extract to *Vibrio harveyii* and *Pseudomonas fluorescence* bacteria found in Southeast Maluku waters.Through a biotechnology-based approach, it is hoped that it can provide solutions to specific problems that are associated with pathogenic bacterial resistance.

MATERIALS AND METHODS

Sample Collection and Preparation: The *S.filipendula* sample, sampled from the waters of Southeast Maluku Kelanit in May 2017 was 3 kg. The samples obtained are then inserted into a plastic bag to be taken to the laboratory. Samples were air dried for ± 14 days with supervision. The dried sample (simplicia) is cut into small pieces and then mashed with a blender until it becomes a simplicia powder.

Extraction: *Sargassum filipendulla* dried seaweed is ground into powder form and then extracted by maceration method. Comparison of samples with solvents is 1: 4 (b / v). (Alamsyah *et al.,* 2014). Blended samples were soaked in 96% ethanol as much as 20 liters *(maeserasi).*Extraction is carried out *(maeserasi)*at room temperature. The filtrate 1,2,3,4,5 and 6 obtained were collected in the conductor then separated between ethanol and extract using rotary evaporator. Then phytochemical testing was carried out to determine the phytochemical content of *S.filipendulla* extract.

Bacterial Renewal and Test Bacterial Suspension: *V. harveyi* and *P. fluorescence* bacteria originating from pure tillers, each of them taken as many as 1 ose which was then inoculated by scratching on a sloping Nutrient Agar (NA) medium. Bacterial cultures on each sloping agar were incubated at 37 0C for 18-24 hours. Test bacteria which have been reneweled, each of them is taken 1 ose then suspended into a sterile Sodium Broth (NB) liquid media, after which they are homogenized. The transmittance measured at 25% using a spectrophotometer as the blank used is Sodium Broth (NB) at a wavelength of 580 nm.

Testing the antibacterial activity of S. filipendula ethanol extract: Testing of inhibitory activity was carried out in vitro with agar diffusion method (Kirby Bauer) using paper discs or papperdisk oxid. This was done using 5 treatment concentrations which were 100%, 75%, 50%, positive control (amoxicillin) and negative contral (solvent) .Observation of the inhibition zone is done by measuring the diameter of the barrier area (clear zone) which is the bacterial growth around the filter paper using a ruler.

Data analysis: The data obtained in this study are the results of phytochemical testing and the results of the calculation of the inhibitory zone diameter in the antibacterial activity test. Data from the measurement of inhibition zone diameter were then analyzed using RAK Factorial analysis method followed by the Anova test.If there is a different treatment, it will be followed by a Least Significant Difference (LSD) test.

RESULTS

Phytochemical content of *S. filipendulla* extract: Brown algae of *S. filipendulla* produced extracts in the form of green paste to blackish green with extract rendemen values which can be seen in Table 1. The solvents used consisted of 3 different solvents based on their level of polarity namely ethanol solvent, n-hexane solvent and ethyl acetate solvent with technical purity.The algae extract of *S. philipendulla* obtained and tested the phytochemical content quantitatively to determine the compounds contained in the extract. The results of phytochemical testing of *S. filipendulla* extract are presented in Table 2

Antibacterial Activity of *S. filipendulla* Extract: Antibacterial activity of brown algae extract of *S. filipendulla* against pathogenic bacteria *Vibrio harveyii* and *Pseudomonas. fluorescence* are seen in Table 3. The results of testing the antibacterial activity of *S. filipendulla* extract on *Vibrio harveyii* and *Pseudomonas fluorescence* bacteria are shown in Table 4. The results of the Least Significant Difference (LSD) test were carried out to see the relationship between bacteria, extracts and concentrations used as shown in Table 5 and Table 6, looking at the average value of extracts to inhibit bacteria. The average antibacterial extract to inhibit *V.harveyi* and *P. fluorescence* bacteria areshown in Figure 1.

DISCUSSION

Phytochemical content of *S. filipendulla* extract: *S. filipendulla* extract from Southeast Maluku waters is green to blackish green and in the form of paste.

Table 1. The rendemen of *S. filipendulla*extracts

P	BE (gr)	R (%)	B	W
Ethanol	474,9	15,83	paste	Green
n-Hexan	48,76	12,19	paste	Blackish Green
Ethyl Acetate	2,53	5,19	paste	Blackish Green

Description:
P: Solvent
BE: Extract Weight
R : The rendemen
B : Form
W : Color

Table 2.Phytochemical content of extract of *S. filipendulla*

No	Secondary Metabolites	Test Method	Test Results of Each Extract		
			Ethanol	n-Hexan	Ethyl Acetate
1.	Phenolic	Reactor $FeCl_3$ 5 %	+	+	-
2.	Tanin	Reactor $FeCl_3$ 1 %	+	+	-
3.	Flavonoids	a. Reactor of Concentrated HCl + Mg	-	-	-
		b.Reactor H_2SO_4 2N	+	-	-
		c.Reactor NaOH 10 %	+	-	+
4.	Saponin	Heated up	+	+	+
5.	Triterpenoid	Reactor H_2SO_4Concentrated + CH_3COOH	-	+	+
	Steroids	Anhidrat	+	+	-
6.	Alkoloid	a.Reactor of Dragendorff	-	+	-
		b.Reactor Meyer	-	-	-

Description:
(+) = Exist
(-) = non-Exist

Table 3. Diameter of *S.filipendulla* extraction zone to bacteria of *V.harveyi* and *P. Fluorescence*

Bacteria	Concentration	Average Diameter (mm)			Description
	(%)	Ethanol	n-Hexan	Ethyl acetate	
V. harveyii	100	24 (+++)	9 (+)	12 (++)	Active
	75	-	5 (+)	10 (++)	Active
	50	23 (+++)	-	6 (+)	Active
	Amoxicillin (Control +)	20 (+++)	20 (+++)	19 (++)	Suspectible
	(Control -) Solvent	-	-	-	Not active
P. flourosences	100	14 (++)	7 (+)	13 (++)	Active
	75	11 (++)	-	8 (+)	Active
	50	14 (++)	-	11 (++)	Active
	Amoxicillin (Control +)	14 (++)	14 (++)	14 (++)	Suspectible
	(Control -) Solvent	-	-	-	Not active

Description :
(-) = Not active
(+) = Low activity (7-10 mm)
(++) = Moderate activity (10-15mm)
(+++) = high activity (> 20 mm)

Table 4. Results of Antibacterial Activity Test of *S. filipendulla* Extract To Bacteria of *V. harveyii* and *P. fluorescence*

Source	DF	Sum of Square	Mean Square	F Value	Pr(> F)
repeating	2	4.8222	2.4111	1.07	0.3492
bacteria	1	78.4000	78.4000	34.84	0.0000
exstract	2	620.0889	310.0444	137.79	0.0000
kons	4	3057.8444	764.4611	339.73	0.0000
bacteria: extract	2	29.0667	14.5333	6.46	0.0029
bacteria:kons	4	139.0444	34.7611	15.45	0.0000
extract:kons	8	888.0222	111.0028	49.33	0.0000
bacteria: extract:kons	8	387.4889	48.4361	21.53	0.0000
Error	58	130.5111	2.2502		
Total	89	5335.2889			

Summary Statistics shows results:

CV (%)	Diameter Mean
16.83	8,91

Table 5. Relationship between Bacteria, *S. filipendulla* Extract and Concentration

Bacteria	Concentration (%)	N	Extract		
			Ethanol	n-Heksan	Ethyl Acetate
V. harveyi	100	3	23,6667[a]	9,3333[a]	12,0000[a]
	75	3	0,0000[b]	5,3333[a]	9,6667[a]
	50	3	22,6667[a]	0,0000[a]	6,3333[b]
	Control (+)	3	19,6667[a]	20[a]	19[a]
	Control (-)	3	0,0000[a]	0,0000[a]	0,0000[a]
P. Fluorescence	100	3	14,0000[b]	6.6667[b]	12,6667[a]
	75	3	10,6667[a]	0,0000[b]	8,3333[a]
	50	3	14,3333[b]	0,0000[a]	10,6667[a]
	Control (+)	3	14,3333[b]	14[b]	14[b]
	Control (-)	3	0,0000[a]	0,0000[a]	0,0000[a]

Means with the same letter are not significantly different Alpha 0.05

Table 6.*S. filipendula* Extract to Inhibit Bacteria in Different Concentrations

Concentration (%)	N	Ethanol Extract	N-Hexane Extract	Ethyl Acetate Extract
V. harveyi				
100	3	23.6667 a	9.3333 b	12.0000 b
75	3	0.0000 c	5.3333 c	9.6667 b
50	3	22.6667 a	0.0000 d	6.3333 c
Control (+)	3	19.6667 b	20.0000 a	19.0000 a
Control (-)	3	0.0000 c	0.0000 d	0.0000 d
P. Fluorescence				
100	3	14.0000 a	6.6667 b	12.6667 ab
75	3	10.6667 b	0.0000 c	8.3333 c
50	3	14.3333 a	0.0000 c	10.6667 bc
Control (+)	3	14.3333 a	14.0000 a	14.0000 a
Control (-)	3	0.0000 c	0.0000 c	0.0000 d

Means with the same letter are not significantly different Alpha 0.05

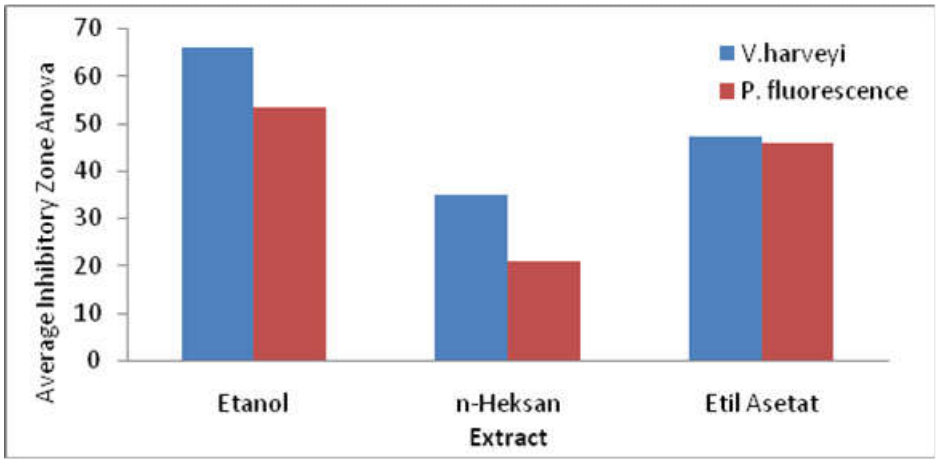

Figure 1. Average Antibacterial Extract

Based on Table 1, the highest rendemenvalue was found in ethanol extract and the lowest was obtained from ethyl acetate extract, indicating that the ethanol extract is more soluble in ethanol solvents and the polar compound content of *S. filipendulla*is more than semipolar and nonpolar compounds. In line with the study (Samee *et al.,* 2009) using methanol, n-hexane and ethyl acetate solvents, the highest value was obtained for methanol (38.86%) which tends to be polar. Phytochemical results of *S.filipendulla* extract showed that phytochemical compounds that were detected were phenolic, tannin, flavonoids, saponins, steroids (ethanol solvents), phenolics, tannins, saponins, triterpenids, steroids, alkaloids (n-hexane solvents) and flavonoids, saponins , triterpenoid (ethyl acetate solvent).The presence of antibacterial compounds on *S. filipendulla* is the result of secondary metabolism which functions as a form of self-defense against unpleasant environments (UV ray), herbivore organisms, fouling organisms, and pathogenic bacteria (Anandhan, 2011).

The results showed that saponin compounds were found in the three solvents used, this was due to the presence of strong glycoside bonds that caused saponins to be polar (5).According to (3), saponin works as an antibacterial by disrupting the stability of bacterial cell membranes, which causes important bacterial components such as proteins, nucleic acids and nucleotides to escape so that the bacteria become lysis.Saponins can be antibacterial because the surface active agents are similar to detergents; consequently saponins will reduce the surface tension of bacterial cell walls and damage membrane permeability (Madduluri *et al.,* 2013). Saponins diffuse through the outer membrane and susceptible cell walls and then bind to the cytoplasmic membrane to disrupt and reduce the stability of the cell membrane; this causes the cytoplasm to leak out of the cell resulting in cell death. Steroid compounds were found in extracts with ethanol and n-hexane solvents. According to (Alamsyah *et al.,* 2014), steroids have

bacterial inhibition mechanisms by damaging bacterial cell membranes by increasing cell permeability, resulting in cell leak followed by the release of interacellular material.Alkaloid compounds are only found in n-hexane extract where these compounds work to interfere with the constituent components of peptidoglycan in bacterial cells, so that the bacterial cell layer is not formed intact and causes cell death in bacteria (Alamsyah *et al.,* 2014). Another mechanism for antibacterial alkaloids is the alkaloid component known as DNA intercellator and inhibits bacterial cell topoisomerase enzymes (Karou *et al.,* 2005). Alkoloid is toxic to microbes so it effectively kills bacteria. besides that alkoloid is able to increase endurance (Alghazeer *et al.,* 2013). Flavonoids act directly as antibiotics by disrupting the function of microorganisms such as bacteria.Its mechanism of action is thought to denaturate bacterial cell proteins and damage cell membranes irreparably. Tanin is a phenol compound that has the properties of alcohol, one of which is antiseptic (a microorganism inhibitor) so that it has the potential as an antibacterial (Alghazeer *et al.,* 2013; Tanniou *et al.,* 2013).

Antibacterial Activity of *S. filipendula* Extract: Table 3 shows that the 100% concentration has the highest inhibitory zone value to inhibit *V.harveyi* and *P. fluorescence* and the lowest inhibition zone at a concentration of 75%.This shows that the higher the concentration of extract used, the greater the inhibitory diameter value. The concentration of 100% has a high inhibition zone because it is the concentration of pure antibacterial extract so that the diameter of the inhibitory zone obtained is the result of the maximum inhibition zone.If the inhibitory zone value is 7-10 mm the inhibitory activity is categorized as weak, if the 10-15mm resistance zone is categorized as medium and if> 20mm is categorized as strong (12).ANOVA test results show a significant result between concentration and extract to inhibit bacteria (Table 4).

Antibacterial compounds found in *S. filipendulla* can inhibit the growth of pathogenic bacteria *V.harveyi* and *P.fluorescence*, with moderate to high inhibitory power. Differences in inhibitory power in each concentration may be caused by extract concentration factors, bacterial sensitivity, and environmental conditions of test bacterial media such as temperature, incubation time, and age of bacteria. This is in line with the opinion (Izzati, 2007) the size of the resistance area is influenced by the growth rate of microorganisms, the ability and rate of diffusion of the active ingredients in the medium, the sensitivity of microorganisms to the active substance and the thickness and viscosity of the medium. the ability of the bacteria itself to fight antibacterial activity is different, depending on the thickness and composition forming the bacterial cell wall.Some studies show that brown algae extract has higher antibacterial activity compared to green algae and red algae (Rajendra *et al.,* 2011). Other studies show that brown algae has the highest antibacterial activity compared to green algae and red algae, this can be seen from the diameter of the inhibitory zone ranging from 10-22 mm (Pangestu *et al.,* 2017). Other studies also tell that sargasum sp extract is effective for suppressing bacterial growth v. harveyi which is indicated by a broad resistance zone (Hwam *et al.,* 2011).

Table 5 shows that ethanol extract has a high value for inhibiting pathogenic bacterium *V.harveyi*and *P. fluorescence* and the lowest is on ethyl acetate extracts whereas positive suspect controls on *V.harveyi* and *P. fluorescence* and negative controls have no effect on pathogenic bacteria. Table 6 provides information on 100% concentration on ethanol extract which gave a significant effect to inhibit pathogenic *V.harveyi* and *P. fluorescence* ($P < 0.05$) when compared with ethyl acetate and n-Hexane extracts.Conversely 50% concentration gives the results of a real difference between ethanol and ethyl acetate extract, at the level of 0.05 ($P < 0.05$) while the n-Hexane extract gives a non-significant result of ethanol and ethyl acetate extract. The highest extract value to inhibit *V. harveyii* bacteria which was the highest in ethanol extract was followed by ethyl acetate extract and the lowest was in n-Hexane extract (Figure 1).Of the three extracts used, ethanol extract is a good extract to inhibit pathogenic bacteria compared to n-hexane and ethyl acetate extract. In line with the research (Ikrom, 2013) about the active substances from Sargassum which are found in both water, methanol and ethanol extracts, and actively inhibit *V.harveyi* bacteria. The results showed that *S. filipendulla* algae extracted using ethanol; n-hexane and ethyl acetate solvents had bacteriostatic activity.Bacteriostatic agents work to inhibit protein synthesis by binding while ribosomes areorganism. The bond is not so strong that when concentration and stability decrease, antibacterial agents release ribosomes so that bacteria can grow back (21).

According to Pelczar and Chan (2005) in (2) each bacterium has a different susceptibility to the physical and chemical properties of antibacterial compounds.Another thing is also due to the nature of the resistance to the antibacterial compounds of the organism.*V.harveyi* and *P. fluorescence* are gram negative bacteria that have a more complex arrangement of cell walls and cell walls composed of an outer membrane consisting of lipopolysaccharide and lipoprotein which function as a barrier to the entry of disinfectants and antibacterial compounds (Telaro *et al.,* 2009), furthermore it is said that antibacterial activity is affected by extract concentration, content of antibacterial compounds, diffusion power of extracts and types of bacteria. The results of this study also showed that the extracts with ethanol solvents showed the best results, it was assumed that ethanol had hydrophilic and lipophilic properties so that the polarity was optimum and the antibacterial substances obtained were maximal (Guedes *et al.,* 2012).

Conclusion

*S. filipendulla*extract from Southeast Maluku waters is in the form of paste, green to blackish green containing phenolic, tannin, flavonoid, steroid, triterpenoid and alkaloid chemical compounds where saponin compounds are found in the three types of solvents used. The resulting inhibitory zone is 24 mm (V.harveyi) and 14 mm (P. fluorescence) and is bacteriostatic. Ethanol extract has a high value to inhibit pathogenic bacterium *V.harveyi* and *P. fluorescence* and the lowest is on ethyl acetate extract whereas positive control is suspect against bacteria and negative control does not affect pathogenic bacteria. The 100% concentration of ethanol extract has an effect on inhibiting the pathogenic bacteria *V.harveyi* and *P. Fluorescence*, the concentration of 50% gives a significant difference between ethanol and ethyl acetate extract, while the n-hexane extract gives a non-significant result of ethanol and ethyl acetate extract. Of the three extracts used, ethanol extract is a good extract to inhibit pathogenic bacteria compared to n-Hexane and ethyl acetate extract.

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