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Antibacterial and Phytochemistrial Activity Test of Brown Macroalgae Extract towards Vibrio Algynoliticus Bactery through *In-Vitro* Fertilization

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Abstract: In the long term this research aims at finding a method to enhance the control of bacterial diseases in a safer and environmentally friendly fish farming. Meanwhile, the special target to attain is to obtain active antibacterial compounds through in vitro fertilization based on solvent polarity level and brown macroalgae active extract test through in vitro fertilization which can be used to increase fish survival rate in controlling bacterial diseases in fash farming. The extraction with methanol solvent results in the highest crude extract in all extracted seaweeds. This is justiciable because the greatest yield is those resulted by methanol solvent. We can see that seaweed species of *Sargassum olygocystum*, *S. cristaefolium*, *S. hemphyllium*, turbinaria ornate and padina australis can produce the highest inhibition zone of 12.0 mm compared to other seaweed species extract in 100% concentration which is then followed by *S. cristaefolium* methanol extract, turbinaria water extract and Padina australis water extract. Based on qualitative test chemical compound content of brown seaweed species, they contain phenolic, flavonoid, steroid and tanin compound. Whilst all qualitative tested seaweed contain no saponin. Thus, it is highly possible to develop those compounds as natural antibacterial and immunostimulant.

Key words: Antibacterial and phytochemistrial, macroalgae, in-vitro fertilization, antibacterial, qualitative

INTRODUCTION

Seaweed contains primary and secondary metabolite. The primary metabolites are vitamin, mineral, fibre, alginate and karaginan to be made as cosmetics substance for skin treatment. Besides its economical primary content, the secondary metabolite content of seaweed can be potentially produced as various bioactive metabolites with wide range of activities such as antibacterial, antivirus, antifungal and cytotastic.

Green, red, or brown seaweed are potential resources of bioactive compound highly beneficial for developing pharmautical industry as antibacterial, anti tumor, anti cancer or as reversal agent and agrochemical industry primarily for antifeedant, fungicide and herbicide (Bijanti, 2005). According to Kordi, seaweed is greatly utilized by coastal communities as external medicine such as natural anticeptic. Research finding of Pringgenies shows seaweed potential as phatogens antibacterial inducing infectional diseases. One of such infectional diseases which commonly infect farmed fish is that causing scarlet fever disease.

Fish illness is one of the obstacles of fish farming industry. This is so because epidemic can trigger massive fish mortality or farmed shrimp. The high rate of farmed fish mortality can decrease fish production which also decreases income as compared to the expenses for fish farming such as fish fry purchasing, fish feed purchasing, fishpond making, wage expenses and et cetera besides, the illed fish will only have lower sale value than that of healthy fish, especially for fresh fish selling.

On the basis of its cause, the fish diseases are divided into two categories namely infecting and non infecting diseases. Infecting diseases are diseases caused by pathogen infection into the wet mother body. The pathogenic diseases of fish are virus, bactery, parasites and fungus. Meanwhile, non infectious diseases are diseases caused by other than pathogenic infection, such as environmental degrading quality, malnutrition and genetical physical defect. All these things can occur either in freshwater fish farming or in sea fish farming.

The control towards bacterial diseases in either freshwater fish farming or sea fish farming has been done all this time by using antibiotics. Antibiotics usage is beneficial when used appropriately based on appropriate diagnosis and dosage, easy to obtain and has stronger and faster visible effect. However, consistent antibiotics application can trigger resistance, leave residue in fish

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body and pollute the environment which then can poison non targeted organism. Therefore, it is highly important to search other safer and more effective and environmentally friendly method to control fish diseases at safe level. *V.alginolyticus* and *aeromonas hydrophila* bacteria are one of pathogenic found in both sea water and freshwater causing fish diseases and death. One of alternatives to solve the problem is by utilizing active compound of brown macroalgae which are safe and environmentally friendly.

One of the succesful diseases control attempt is the usage of antimicrobe compound made of marine algae using crude extract of padina australis which is given by effective immersion in mouse seafish medication which are affected by *V. alginolyticus* bactery by increasing mouse seafish survival rate to 100%. It is greatly assumed that the extract contains phenol compound which are lethal for *V. alginolyticus* bactery. It is stated by Xiao-Jun, Chkhikvishvili and Ramazanov (2000) that *Sargassum furcatum*, *Dictyota* sp, *Sargassum desfontrainessi*, *Padina pavonica* and other kind of brown algae contain phenol compound such as florotannin a kind of tannin which is potential for antibacterial.

On the basis of this research, it is highly assumed that brown macroalgae is highly potential to be made as natural medicine substance in controlling vibriosis and aeromonas disease. Thus, it is necessary to conduct antibacterial activity test with the lowest dosage of several kinds of brown algae in South East Maluku water area which has never been analyzed academically to obtain bioactive compound which can increase fish survival rate and antibacterial activity in controlling fish disease.

Special research objective: The general objective of this research is to find safer and environmentally friendly bacterial disease contol method in fish farming which can function as medicine in disease control of fish seed by using some brown algae. Whilst, the special research objectives are. Knowing the appropriate solvent based on polarity level to obtain bioactive compound of brown algae *Padina australis*, *Padina tetrastomatika*, *Sargassum policystum*, *Sargassum cristaefolium*, *Dictyota dichotoma*, *Turbinaria ornata*, *Turbinaria decurren*, *Hydroclathrus clathratus* which function as antibacterial.

Searching for active compound of brown algae *Padina australis*, *Padina tetrastomatika*, *Sargassum policystum*, *Sargassum cristaefolium*, *Dictyota dichotoma*, *Turbinaria ornata*, *Turbinaria decurren*, *Hydroclathrus clathratus* which have antibacterial quality and can be used in fish farming safely and environmentally friendly.

MATERIALS AND METHODS

The research can be done in two stages. The first stage aims at extracting active compound of *Padina australis*, *Padina tetrastomatika*, *Sargassum policystum*, *Sargassum cristaefolium*, *Sargassum olygocystum*, *Dictyota dichotoma*, *Turbinaria ornata*, *Hydroclathrus clathratus* which are antibacterial to obtain the lowest concentration of the active compound to resist *V. alginolyticus* bactery and *A. hydrophila* through *in vitro* fertilization. In order to know the chemical compound of brown algae, we conduct phytochemical test which is antibacterial for fish. The whole steps of the first stage is conducted in basic chemical laboratory and pathological laboratory in state fishing polytechnic.

Extraction, phytochemical active compound of brown algae shows antibacterial characteristic and resistability test towards *V. alginolyticus* and *A. hydrophila* bacteries through *in vitro*. Sample collection and extraction of brown algae. At this stage, the researcher conducts several steps including sample preparation and active substance extraction. At the preparation stage, brown algae is taken in dry condition in South East Maluku and crushed by cutting them into tiny parts into powder. The next step is active substance extraction. The method used for extraction is Harbon method in 1987 which has been modified using three kinds of solvents based on its polarity level.

Antibacterial test: The three produced thick extract (extract n hexena, dycloromethan and methanol) undergo antibacterial test to find which extract can actively resist bactery. This test use disk testing where sterilized disk paper is immersed in each extract. After 15-30 min or some, the disk will be attached to TCBS media which has been innoculated with *V. alginolyticus* bactery. The measurement is conducted during incubation period for 24 h at 25°C by observing the exustence of clear zone formed around the disk paper.

Phytochemical test of brown algae species potential As antibacterial

Alkaloida compound (culvenor-fitzgerald method): The 4 grams of brown powedered algae is crushed by using crusher, then little chloroform is added until it become pasta. Afterwords, 10 mL of ammoniac-choloroform of 0,05 N is added and is crushed again, the layer of 10 mL H2SO4 2N is formed and strongly shaken. Then, it is cooled until it forms two layers. Then, a fllece of cotton is inserted in the tip of pipette to filter it. Sulphate acid layer is taken and poured into small reaction tube. Philtrate is tested by mayer reactor. The formation of white deposit with mayer reactor shows the existence of alkaloid.

Flavanoid compound analysis (shinoda method cyanidin test): About 0.5 mg powdered sample is extracted with 5 mL methanol and is heated for 5 min in tube reaction. The extract is added with some drops of thich HCL and a little bit of magnesium powder. If it change color into red or yellow it means the extract contains flavonoid.

Saponin compound analysis (foam test): For the saponin test it is suggested to use dried sample because the test used is foam test. The dried sample is crushed and is poured into reaction tube and is added with 10 ml distilled water and is boiled for 2-3 min. Afterwords it is coolded and shaken powerfully. Constant foam for 5 min means an existence of saponin content.

Polyphenol coumpound analysis: mL extract (ethanol, n-hexan-ethanol) is added with FeCl3 1%. Terpenoida compound is signed with the emeregence of blue color, black, or purple. Terpenoid and steroid analysis (lieberman-burchard method) several drops of choloroform in alkaloid test is placed on drop plates and is added with 5 drops of anhydride acetat then is dried and added with 3 drops of thick H2SO4.

The observed parameter: The observed parameter in this research is clear zone diameter of each extract and chemical content of various species of brown algae.

RESULTS AND DISCUSSION

Extraction of active substance in brown algae. The extraction result of brown algae can be seen in Table 1. The resulted extract from extraction process of various species of brown algae differ variously based on solvent kind used such as methanol extract, ethyl acetat extract and extract n-hexan with comparison (1:3). The yield of each extract can be seen in Table 1. Yield is the comparison of extract weight resulted with first weight of used substance and is stated in percentage (%). Extraction of brown seaweed. Extraction with methanol solvent can produce the highest crude extract in all extracted seaweed. This is justiciable bacause the highest yield produced is yield produced with methanol solvent. This is in line with the statement stating that methanol can extract organic compound, some part of fat and tannin causing great methanol extract (Heath and Reineccius, 1987). The extraction result is affected by several factors namely natural condition of natural resources, extraction method, size of particle sample and condition and length of sample storage. This is so because during maceration there is a mixing of extracted substances which enlarge the possibility of collision between particles causing cell splitting with the hope that the expected component can come out of network substance and dissolve in the solvent and in order to magnify fastening and reaction between active substance component with used solvent (Gaspez, 1991).

Table 1: Yield result of crude extract of sargasum cristafolium, ethyl acetat and n-hexan

Kind of RL	Dried weight (gram)	Rendemen (%) Methanol	Ethyl acetat	n-hexan
S.cristaefolium	100	6.91	1.50	1.0
S.polycystum	100	11.71	3.70	2.7
S.hemiphyllum	100	9.80	5.21	2.5
P.tetrastomatica	100	8.61	4.53	2.4
P.australis	100	9.70	6.40	3.5
Turbinaria ornata	100	8.50	3.40	2.5

Table 2: Phytochemical test of brown algae species crude extract

Secondary/metabolit	Test method	A	B	C	D
Phenolic	$FeCl_3$ 5% reactor	+	+	+	+
Flavonoid	Thick HCl+Mg reactor	-	-	-	-
	H_2SO_4 2N reactor	+	+	+	+
	NaOH 10% reactor	+	+	+	+
Steroid	Lieberman-burchard reactor	+	+	+	+
Triterpenoid		-	-	-	+
Saponin	HCl+H_2O reactor	-	-	-	-
Tanin	$FeCl_3$ 1% reactor	+	+	+	-

Explanation:A = Ethyl Acetat extract of sargasum cristaefolium; B = Hexana extract of sargasum cristaefolium; C = Ethyl Acetat extract of sargasum olygocystum; D = Hexana extract of sargasum olygocystum

Table 3: The result of clear zone towards vibrio alginoliticus (Cm) Bactery

Kind of substance	Concentration (%) 15	25	50	75	100
Sargassum. Methanol 1:4	-	-	-	-	-
Sargassum cristafolium	-	-	-	-	-
Sargassum olygocystum methanol	-	-	-	0.6	1.00
Sargassum oligocystum. N-Hexan	-	-	-	-	1,20
Sargassum. hemiphyllum N-Hexan	-	-	-	-	-
Turbinaria ornate aquades	-	-	-	0.6	1.00
Padina australis aquades	-	-	-	0.65	1.00
Padina tetrastomatica	-	-	-	-	-

Phytochemical test of brown seaweed extract: To develop *A. acuminata* as antibacterial and immunostimulant, it is suggested to know the chemical compound of the extract from several kinds of seaweed extract. The qualitative test of chemical compound content extract of brown algae.

Based on the chemical compound content qualitative test of various brown seaweed as shown in Table 2, it is known that all kinds of brown seaweed as seen in Table 3 we can tell that all kinds of brown seaweed contain phenolic, flavonoid and steroid, steroid and tannin compound. Whilst, all kinds of qualitatively tested brown seaweed contains no sapponin. Therefore, it is highly possible that these compounds develop as antibacterial and natural immunostimulant.

According to Satria (2005), flavonoid compound can function as antioxidants by resisting kinds of oxidation reaction and can reduce hydroxyl,

superoxide and radical peroxyl. Antioxidant compound captures free radicals, metal solidify and singlet oxygen formation reducer.

Phenol compound can also change surface tension which damages selective permeability of microbe membrane cell producing essential methabolic and inactivating bacterial system. The damage of this membrane enables neuclotide and amino acid to come out of cell. Besides, the damage can also prevent the intrusion of essential substances into the cell because the membrane cell also controls active transportation into the cell. This can cause bacterial cell death or resist bacterial development (Volk dan Wheeler, 1988).

Tannin compound contained in Widuri leave also can function as antimicrobe. Kim and Fung state that tannin can formulate complex protein with protein and hydrophobic interaction. When hydrogen and tannin and protein are bound, it is possible that the protein will be sedimented. This phenomenon is well known as protein denaturation. When enzyme protein of microbe is denaturated, the enzyme will be inactive so that the microbe metabolism will be disturbed which will cause cell damage.

Steroid working mechanism in impeding microbe is by damaging plasm membrane which leak out cytoplasm cell and leads to cell death (Putra, 2007). Afterwords, according to Cowan (1999), phenolic compound is an antibacterial which disturb cytoplasm membrane function. Phenol is also a compound of OH group bound in aromatic ring. Phenolic is also secondary metabolite spread in plants. Phenolic compound in plants can be in the form of simple phenol, anthraquinon, phenolat acid, coumarin, flavonoid, ligin and tannin.

Antibacterial activity test from brown seaweed extract towards vibrio algynoliticus and aeromonas hyhrophila bactery through *in-vitro* fertilization. In Table 3, we can tell that not all extract can impede *A. hydrophila* bactery. It is known that *Sargassum olygocystum*, *S. cristaefolium*, *S. hemphyllium*, *Turbinaria ornate* and *Padina australis* seaweed species can produce resisting zone eventhough, each extract produce different resist zone. N-hexan extract of *Sargassum olygocystum* has the greatest resist zone of 12.0 mm compared to other seaweed extracts in 100% concentration. Then, it is followed by *S. cristaefolium* methanol extract, turbinaria water extract, *Padina australis* water extract.

S. olygocystum N-Hexan extract has the highest resistance because, this extract contains phenol, flavonoid, steroid triterpenoid and tannin which collaborate to resist *V. algynolyticus* bactery through *in vitro* fertilization. However, this resistance is still categorized as mild category. This is according to resist zone criteria for bactery according to Stout as referred in Rachdiati who states that resistance zone with 5-10 mm average is included in mild criteria. Meanwhile, resistance zone of <5 mm is included in weak criteria. This is caused by different ability of each extract with different quality and quantity content and different media extract absorption system so that the number of various bactery and patogenity level result in different resistance zone.

The resistability or killing power of an antimicrobe is greatly affected by many factors such as concentration factor. Darkuni states that the ability of an antimicrobe substance which can abolish the ability towards certain microorganism depends on the concentration of antimicrobe substance. In other words, antimicrobe substance in a bacterial environment greatly determine the bactery survival ability. Therefore, there are certain bactery which can survive and even have active metabolism in an antimicrobe environment. Volk and Wheeler (1988), state that the main factor to determine how antimicrobe substance can work effectively is contration, duration for the substance to work, temperature and number of species and microorganism.

Mallawa and Halid state that the measurement of growth resistance zone becomes the standard of bioactivity which is affected by many factors such as function group activity, bacterial resistance of bioactive substance, active substance level and tsted bacterial density.

CONCLUSION

Based on the result and analysis of the research, we can conclude that:

- All extracts can produce resistance zone. However, each extract produces different resistance zone, except that of water extract Padina tetrastomatica, which has no resistance power in all concentration
- Based on qualitative test, the chemical compound content of many kinds of brown seaweed, we can tell that all kinds of brown seaweed contain phenolic, flavonoid and steroid, tannin and all kinds of qualitatively tested seaweed contain no saponin. Thus, it is possible to develop these compounds as antibacterial and natural immunostimulant.

SUGGESTION

It is necessary to develop further research about the kinds of brown algae with appropriate dosage as antibacterial active compound towards *V. algynoliticus*, *A. hidrophila* in tiger grouper fish and tilapia fish at laboratory scale.

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