BACTERIA EXTRACT ACTIVITY ASSOCIATED WITH SPONGES *Haliclona* sp.2 AND *Axinellid* sp. AS ANTIBACTERIAL.

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**Abstract**

Isolation of bacteria for antibacterial activity was conducted on sponge-associated marine bacteria, *Haliclona* sp.2 and *Axinellid* sp. from Ngge Island, Sibolga. Seventeen isolates were obtained from the two sponges. Based on antibacterial test, 11 isolates (64%) were potential as antibacterial, whereas 6 isolates (35%) were not indicated as antibacterial. Isolate H2, A1, and A2 with broad spectra of antibacterial activity were further separated for extraction of secondary metabolites. Based on screening results of isolates, ethyl acetate extract of isolate H2, A1, and A2 and n-hexana extract of isolate A2 displayed antibacterial against *Staphylococcus aureus* and *Escherichia coli*, whereas methanol extract A2 was only able to inhibit the growth of *Escherichiacoli*. For bioactive compound test, EH2, EA1 and EA2 were positive for alkaloid, whereas HA2 and MA2 were positive for alkaloid and saponin. Based on purification of secondary metabolite using Thin Layer Chromatography (TLC), there were 22 fractions with solvents of different polarities. Screening of antibacterial activity for isolate extract fractions showed that MA2 of fraction 2 had a strong inhibitive effect against *E.coli* and *S. aureus* about 10,15 mm and 9,95 mm consecutively. The antagonistic activity of EH2of fraction 3 against *S. aureus* and *E.coli* were about 7,05 mm and 7,45 mm consecutively, whereas EA1 of fraction 3 was about 6,55 mm against *P. aeruginosa*. The results of phytochemical secondary metabolite of isolate extract fractions showed that MA2 of fraction 2 and MA2 of fraction 3 werepositive for saponin, while EH2 fraction 3 and EA1 fraction 3 were positive for alkaloid. Bacteria associated with sponges *Haliclona* sp.2 and *Axinellid* sp. had activity as antibacterial against bacteria *S. aureus*, *E. coli* and *P. aeruginosa*.

**Introduction**

Pathogenic microba has been considered as a crucial problem in human healthy and can cause various diseases. The use of chemical compounds that acts as antibacterial such as unrationnal antibiotic to treat various diseases caused by pathogen, could make a negative side effect even it could cause bacteria becomes resistance againsts specific...
antibacterial. In order to overcome this issue, it is important to explore antibacterial originated from bioactive compounds of plants, animals and microorganisms.

Sponge is one of corral reef components that has a potential bioactive but not has been explored. Exploration of sponges as bioactive source has been reported in various researches. However, the use of secondary metabolites produced by bacteria associated with sponges as antibacterial source is more beneficial as compared to isolation from its host. The excessive exploration of sponges to produce new bioactive could cause overfishing that could damage marine ecology. In addition to the growth of sponges that is relatively slow will inhibit the biomass availability as source of secondary metabolites. Therefore, the use of bacteria associated with sponges will be efficient because the bacteria could be cultured in laboratory and could be multiplied in relatively fast (Abubakar et al., 2011).

Capability of sponges in producing bioactive compounds due to its symbiosis with bacteria. The relationship sponges with bacteria will produce secondary metabolite as a form of defense against natural enemies such as predators, pathogenic microorganisms, competitor and fouling organisms (Taylor et al., 2007). This relationship includes a generous supply of nutrients through metabolism translocation such as nitrification, nitrogen fixation, photosynthesis, and an enhancement of chemical defenses. Due to the role, symbiotic bacteria with sponges is estimated has a great potential on the production of bioactive compounds isolated from sponges (Kennedy et al., 2008).

Some researchers of marine explore a new bioactive compounds from sponge-symbiotic bacteria. Peptide was isolated from both sponge Hyatella sp. and an associated Vibrio sp. that has antibacterial activity (Taylor et al., 2007). New metabolites from sponge-symbiotic bacteria contribute to has antibacterial and antiplasmodial activity. Extract of n-hexane and ethyl acetate from 4 (four) potential species of bacteria isolated from sponges have antibacterial activity (Dash et al., 2009; Inbaneson et al., 2012). One of antibacterial compounds was isolated from bacteria Pseudovibrio spp. associated with sponges Axinella dissimilis (Halloran et al., 2011).

Bacteria associated with sponges include representative of the Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria (Taylor et al., 2007). Selvin et al., (2009) reported that potential microbe which produce active compounds are Cyanobacteria, Fungi and Actinomycetes. The active compound resulted by sponge Dendrilla nigra associated with Actinomycetes Nocardiopsis dassonvillei MAD08 was as antimicrobial compound against gram-negative bacteria (Staphylococcus aureus MTCC 2940, Staphylococcus aureus MTCC 96, Micrococcus luteus MTCC 106, Rhodococcus rhodochrous MTCC 265) and gram-positive bacteria (Vibrio cholerae PC6, Pseudomonas aeruginosa MTCC 2453 and Escherichia coli MTCC 2939). Some bacteria isolates associated with sponges Jaspis sp. are antibacterial because of inhibiting the growth of Staphylococcus aureus, Vibrio harveyi, Escherichia coli, Pseudomonas aerogenosa and EPEC K-11 (Abubakar et al., 2011). Correlation between production of antibacterial compound by bacterial symbiont with sponges has been investigated by Narsha and Anil (2000), that antibacterial compound produced by bacterial symbionts such as proteobacterium MBIC 3368, Idiomarina sp. and Pseudomonas sp. were very affected by protein recombinant of Subertias domuncula as host. This research confirmed that there is a collaboration in biosynthesis of secondary metabolite between symbion microbe and sponges. Based on various results research, an investigation for sponge-associated bacteria as antibacterial which easy to be cultured, was needed to be conducted. The isolation results will be tested for antibacterial activity against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.

Materials and Methods:-
2.1 Sample collection
The marine sponges were collected off Nggel Island, Sibolga by SCUBA diving at 2-10 m. Temperature, salinity, and pH were determined at the moment of sample collection. Sponges samples were collected as needed then put into plastic bag filled marine water and oxygen then put into cool box. Sponges were identified at Laboratory of Marine Biology of Fishery Faculty of Riau University.

2.2 Extraction of Secondary Metabolite from Potential Bacterial Isolate
Suspension was made with turbidity justified with Standard of McFarland 10⁵ CFU/ml, then spreaded with cotton swab on the surface of NA media. Some suspensions were made on petri disk and incubated for 5-6 days at 25°C. Bacterial cultures which obtained were cut into small pieces then put into erlemeyer 500 ml then it was added ± 150 ml solvent n-heksana, macerated for 3 x 24 hours. It was filtered with Whatman 42, thus resulted macerat and residual. Residual was reextracted with ethyl acetate and filtered. With the same way, residual was extracted with methanol. Each macerat was centrifuged with 3000 rpm for 15 minutes, thus result supernatant. Then the
supernatant was separated with each their solvent by using rotary evaporator at 45°C. It was obtained extract n-heksana, ethyl acetate and methanol secondary of potential bacterial isolate(Nofiani et al., 2009). The series levels of extraction was shown in appendix 4a.

2.3 Screening Bacterial Extract for Antibacterial Activity

Bacterial suspension of Staphylococcus aureus, Pseudomonas aeruginosa and Escherchia coli were as tested bacteria. For extract activity test, suspension of test bacterial culture was prepared by inoculation each them into nutrient broth media and incubated for 24 hours at 25°C. Activity test conducted by inoculated 200µl suspension of bacterial test that was rejuveniled into agar media and spread out with hockey stick thus test bacterial suspension mix on media surface. Then it was kept for a few moment to let the bacterial test fuse with the media. Then it was put into oxoid disk that was drooped with 10 µl of extract –n- hexana secondary metabolite potential bacteria into agar media that was inoculated with Staphylococcus Aureus, Pseudomonas aeruginosa and Escherichia coli. Then incubated for 24 hours at 25°C and investigated inhibition zone formed with vernier caliper. The same treatment was applied against extract ethyl acetate and methanol of secondary metabolites of potential bacteria.

2.4 Identification of Chemical Compound in Potential Isolate Extract n-Hexana, Ethyl acetate and Methanol.

Preliminary test of secondary metabolite was carried out on each potential isolate extract. 5 ml aquadest and chloroform were added into the extract then mixed strongly and let it for a few minute until two layers formed. Water layer was used for metabolite secondary assay of flavonoid, fenolik, and saponin whereas Chloroform layer was used for terpenoid and steroid (Harbone, 2006).

2.5 Identification and Isolation Secondary Metabolite Potential Isolate Extract by Preparative Thin Layer Chromatography.

Chamber was saturated with raising solvent by covered chamber with filter paper, then raising solvent was put into chamber until all filter paper throughly wet by the solvent. Extract solvent contained secondary metabolite of potential isolate spotted preparative TLC with 20 x 20 cm until the plate saturated by extract solvent (1.5 cm from under line), plate was let until dry for ±15 minutes. Previous plate was marked upper and down limit. Then plate put into chamber that was saturated and eluted until the solvent reached upper limit line. Node formed was checked under UV light and pattern formed of node was drawn with pencil. Then the pattern was scraped with spatula and each node was separated into vial furthermore was washed and separated from its silica. Compounds that were separated from the silica, the solvents was volatiled and antimicrobe test was conducted.

2.6 Screening of Antibacteria Activity Fraction of n-hexana, Eti Acetate and Methanol of Potential Bacteria Isolate.

Bacterial suspensions of Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli were as bacterial test. For screening, the suspensions were inoculated nutrient broth media and was incubated for 24 hours at 25°C. Identification of antibacterial activity was used disc diffusion assay(Bauer et al., 1996). 200 µl suspensions that already rejuveniled were inoculated into nutrient agar media and spread out with hockey stick, thus bacterial suspension test was mixed throughly on surface media. Then it was waited for a few minute until the bacterial suspension fused with media. Then it placed on the empty oxoid disk that already spotted with 10µl fraction n- hexana secondary metabolite of potential bacteria into media NA that was inoculated with bacteria S. aureus, P. aeruginosa and E. coli. The culture was incubated for 24 hours at 25°C and inhibition zone formed was observed with vernier caliper. The same treatment was applied on fraction ethyl acetate and methanol of secondary metabolite of potential bacteria. Furthermore, result of screening antibacterial activity was tested for phytochemical identification of potential isolate extract fraction.

Results and Discussions:-

3.1 Sponges Characteristic

Sponges were taken from Ngge Island, Sibolga from 2- 10 m with purposive sampling method by scuba diving. Determination of pH with pH – meter showed that pH of marine water of sponge sampling was 7.5; salinity 30‰ and temperature 29°C. Collecting sponges was located based on GPS that were N. 01. 39. 001. E.098.47.661. N. 01. 34. 379. E. 098. 45. 251. N. 01.34. 361. E.098. 45. 252. N. 01. 35. 419. E. 098. 41. 831. Measurement of sampling location condition was to create suitable environment for symbiont bacterial test thus symbiont bacteria was able survive to live. Sponges sample were taken as needed then put into plastic bag that filled with marine water and oxygen and then put into cool box. The sponges from Ngge Sibolga were Haliclona sp.2 and Axinellid sp.
In addition, *Haliclona* sp.2 has oscula on each branch and blue colour, whereas *Axinellid* sp. has a shape like fan, yellowness, and oscula on whole surface (Gosliner et al., 1996).

![Image of sponges](image)

**Figure 4.1.** Sponges (a) *Haliclona* sp.2 and (b) *Axinellid* sp.

### 3.2 Isolation and Purification of Symbiont Bacteria

Based on isolation results, there were 17 isolates from mesophyl of the two sponges that consisted of 8 isolates of *Haliclona* sp.2 and 9 isolates of *Axinellid* sp. (Appendix 2b). Bacteria in mesophyl was bacteria that has a similarity secondary metabolite with the sponges. Thiel et al. (2007) had discovered bacteria in large amount on sponges *Chondrilla nucula*. The bacteria was isolated based on morphology, size and shape of colony. Seventeen isolates were isolated based on color and colony shape (Table 11). It showed possibility many differences of activity for each isolate that used for further test. Isolation and purification of symbiont bacteria could be shown as follows:

| Species      | Isolate Code | Colony form | Color           | Elevation | The edge of the colony |
|--------------|--------------|-------------|-----------------|-----------|------------------------|
| *Haliclona* sp.2 | H1           | Round       | Yellow          | Flat      | Entire                 |
|              | H2           | Round       | Beige           | Flat      | Entire                 |
|              | H3           | Bobbin      | Beige           | Raised panel | Round               |
|              | H4           | Round       | Yellow          | Flat      | Wave                   |
|              | H5           | Round       | Beige           | Flat      | Entire                 |
|              | H6           | Bobbin      | Yellowish beige | Arise     | Entire                 |
|              | H7           | Round       | Beige           | Flat      | Irregular              |
|              | H8           | Irregular   | Beige           | Flat      | Curly                  |
| *Axinellid* sp. | A1           | Round       | Beige           | Flat      | Wave                   |
|              | A2           | Bobbin      | Yellow          | Arised panel | Entire               |
|              | A3           | Irregular   | Beige           | Panel     | Curly                  |
|              | A4           | Round       | Beige           | Flat      | Entire                 |
|              | A5           | Round       | slightly yellow | Flat      | Entire                 |
|              | A6           | Round       | Beige           | curve     | Entire                 |
|              | A7           | Root like   | Beige           | Flat      | Curly                  |
|              | A8           | Irregular   | Beige           | Flat      | Jagged                 |
|              | A9           | Irregular   | Beige           | Flat      | Blade                  |

Various microorganisms associated with sponges. For instance, unicellular algae, Cyanobacteria, dinoflagellates and Archaea. Among these microorganisms, bacteria are the most dominant that constituted up to 40% of the biomass or 60% of the tissue volume of certain sponge species (Lee et al., 2006). According to Vasanthanabharathi et al., (2012), bacterial density that associated with sponges *H. cribriformis, S. carnosa, C. diffusa* and *S. officinalis Var. ceylonensis* was in the range was $13 \times 10^3 - 1.6 \times 10^7$ CFU/g, $6.77 \times 10^3 - 1.5 \times 10^7$ CFU/g, $7.68 \times 10^3 - 1.1 \times 10^7$ CFU/g and $2.69 \times 10^3 - 1.4 \times 10^7$ CFU/g. In Taylor et al. (2007) described that associations between sponges and microorganisms can be maintained by two ways that is filter feeding which microbes can be recruited from the surrounding water to the sponges (horizontal transmission) or by reproductive stages (vertical transmission). In vertical transmission, oocyte from the adult mesohyl of sponge can phagocytosis to the microbial cells so bacterial symbiont could be found on embryol sponges larva.

Differentiation of diversity and number of bacterial isolates of each kind of sponges might be due to isolation technic and growth media that used for isolation bacteria associated with sponges. Nutrient contents of culture media would affect bacterial growth. According to Haloran et al. (2011), there were 73 from the marine sponges.
Axinella dissimilis, Polymastia boletiformis and Haliclona simulans and identified as being Pseudovibrio spp. by 16S rRNA genes sequencing and phylogenetic analysis. Radjasa et al. (2008) reported, there were 32 bacterial isolates from sponges Haliclona sp. in North Java Sea. Murniasih and Rasyid (2010) also reported that there were 75 bacterial isolates from sponges Theonella sp., Aaptos sp., Melophlus sarasssinorum, Callyspongia sp., Ircinia sp., Stylissa flabeliformes, Lisoclinitum sp., and Clarithria sp. by direct plating. Abdullah (2006) also had obtained 7 bacterial isolates associated with sponges Axinella sp. by using marine broth agar media.

Symbiont bacteria produce a wide array of secondary metabolites as antibacterial (Lee et al., 2001). Although the mechanism of association between sponges and bacteria is not known well, the association could produce secondary metabolite. Secondary metabolite production by sponges has similarity with microorganisms associated with sponges itself, include bacteria (Radjasa et al., 2007). In addition, bacteria associated with organisms have probability an interaction with the host organisms (Lee et al., 2001). The biochemical interaction make a possibility that symbiont bacteria produces the same bioactive compound with its host. In Taylor et al. (2007), was explained that bacteria symbiosis with sponges are able to photosynthesis, oxidize methane, nitrification, nitrogen fixation, sulphat reduction and dehalogenase.

3.3 Extraction of Secondary Metabolite from Potential Bacteria

Extraction of secondary metabolite was carried out by maceration (Appendix 4b). In Nofian et al. (2009) was described that maceration can produce intracellular antimicrobe compound thus bacterial cell need to be broken to obtain antibacterial compounds. Different polarity of solvents in maceration were n-heksana (nonpolar), ethyl acetate (semipolar) and methanol (polar). According to Dash et al. (2009) polarity differences on solvent used in order to isolate secondary metabolite and identification of following stage.

Extraction of secondary metabolites with different polarity of solvent was conducted serially. The isolates for this stage were determined based on the ability to inhibit some bacteria tests, that were H2, A1 and A2. Then, results of secondary metabolite extraction were extract of n-hexana, ethyl acetate and methanol of potential bacteria isolates. Taylor et al. (2007) described that, secondary metabolites produced by symbiont bacteria is potential as precursor of biosynthesis of sponges metabolite.

3.4 Screening Activity of Extract Antibacteria of Isolate Potential Bacteria

Results of screening for antibacterial activity of potential bacteria isolate extract were each isolates of extract ethyl acetate (EH2, EA1 and EA2) and extract n-hexana A2 showed antagonistic capability against bacteria test S. aureus and E. coli.

Figure 3. Sampling of antibacterial activity (a) extract ethyl acetate of isolate H2 (EH2) against S. Aureus and (b) Extract n-hexana, ethyl acetate and methanol of isolate A2 (HA2, EA2 and MA2) against E. coli. Based on screening results of antibacterial activity, H2 and A1 were able to inhibit growth of P. Aeruginosa, while screening results of secondary metabolite extract showed that both isolates did not perform antibacterial activity against P. aeruginosa (Table 4.3). Extract hexana and ethyl acetate of isolate A2 (HA2) resulted inhibition zone against S. aureus den E. coli (Table 4.3) but not for P. Aeruginosa. For antagonist test of isolate showed that isolate A2 was capable of inhibiting growth of both bacteria S. aureus and E. Coli (Table 4.3). According to Tinambunan et al. (2012) extract of bacteria associated with sponges with concentration 10% showed largest zone inhibition of antibacterial activity against S. aureus and E. coli. Extract ethyl acetate ethyl acetate of isolate A2 (EA2) had brospectrum activity because it was capable of inhibiting gram positive and gram negative bacteria and according to David and Stout (1971) it was included as active compound with strong inhibition capacity (diameter of inhibition zone against S. aureus and E. coli were 20,05 mm and 23,00 mm consecutively).
Extracts of three isolates (H2, A1 and A2) showed negative results against bacteria test P. aeruginosa. While extract MA2 only capable of inhibiting growth of bacteria test E. coli (Table 4.3). This might due to MA2 was suggested contain secondary metabolite that was facilitated to disturb permeability cell membrane of gram negative bacteria thus cell bacteria lysis. In Nofiani et al. (2009) explained that there are some suggestions that caused bacterial isolates did not show antibacterial activity namely (1) isolat has gene code formation secondary metabolite compound but it is not expressed at normal condition and will be expressed when it is induced previously; (2) symbiont bacteria produces antimicrobe compound intracellularly but it does not have antibacterial activity against bacteria test. Radjasa et al. (2007) stated that the production of secondary metabolite from the sponges has similar with microorganisms associated with sponges. It indicate that the production of secondary metabolite from simbiont bacteria is affected by host sponge. Thus when simbiont bacteria is isolated from host sponge, it will affect secondary metabolite as antibacteri.

3.5 Test Chemical Compound of Extract n-Hexana, Ethyl Acetate and Methanol of Potential Isolate
Identification of secondary metabolite content is an important initial step in searching new bioactive compound from natural material and as precursor for synthesis new medicine or as a medicine prototype with certain activity (Harborne, 2006). Capability of antagonistic activity against bacteria test is due to presence of secondary metabolite produced by isolate of symbiont bacteria. Test component of extract chemical compounds, n-hexana, ethylacetate and methanol of isolate potential bacteria was conducted as initial test to investigate the characteristic of secondary metabolite. Alkaloid was positive for each extract ethylacetate isolate (H2, A1 and A2) by used dragendorf reagent and resulted red sediment (Appendix 5b). Furthermore, extract n-hexana and methanol of isolate A2 were positive contained alkaloid and sapoin (Table 4.4). Alkaloid and sapoin indicated antagonist ability against bacteria test (Tabel 4.3) whereas identification other secondary metabolite such as steroid, terpenoid, fenolic and flavonoid showed negative result.

3.6 Identification of Secondary Metabolites in Potential Bacteria Isolates by using Thin Layer Chromatography (TLC) Preparative
Identification secondary metabolite compounds of symbiont bacteria was conducted with TLC (Penesyan et al., 2011). Based on preparative TLC results were found 22 fractions observed under UV from five extracts (EH2, EA1, EA2, HA2 and MA2) with different polarity. Movement phase was used to eluted the samples and variated based on polarity gradient (Yuherinta, 2011). Movement phase drained through static phase and carried components of extract potential bacteria isolate. Different component will move with different rate. Based on preparative chromatography results, separation of compounds in extracts of bacteria isolates into pure substances were obtained 6 fractions in HA2 (nonpolar) while EH2, EA1, EA2 and MA2 (semipolar and polar) obtained 4 fractions for each. (Figure 4).

Figure 4. Results of Thin Layer Chromatography (TLC) preparative for secondary metabolite of extract potential bacteria isolate. (a) HA2; (b) EH2; (c) EA1; (d) EA2; (e) MA2.

3.7 Screening Antibacteria Activity on Extract Fraction of Potential Bacterial Isolate
Secondary metabolite fraction of extract potential bacterial isolate showed antagonist ability against bacteria test. Based on screening results of antibacterial activity, the largest antagonist ability of secondary metabolite extract
Antibacterial activity was also found on EH2F3 against bacteria *S. aureus* and *E. coli*. Isolate H2 was obtained from sponges *Haliclona sp.*2 with secondary metabolite alkaloid. Antagonist ability was indicated with the formation of clear zones against bacteria *S. aureus* and *E. coli* with diameter 7.05 mm and 7.45 mm consecutively. Research about antibacterial activity of sponges genus *Haliclona* was also conducted previously. Radja et al. (2007) stated that symbiotic bacteria of sponges *Haliclona sp.* showed antibacterial activity against some bacteria test, one of them was *S. aureus* with diameter > 9 mm. 

Screening results of antibacterial activity of extract fraction of Isolate EA1F3 showed clear zone with diameter 6.55 mm against *P. aeruginosa*. This result was different from antibacterial activity screening of isolate extract, where extract isolate EA1 did not have antibacterial activity against *P. aeruginosa*. Extract fraction screening result of isolate MA2 was also showed differences from extract screening of bacterial isolate. For initial screening, isolate MA2 was only showed antagonistic ability against bacteria *E. coli* whereas extract fraction screening of bacterial isolate MA2F2 and MA2F3 showed antagonistic ability against *E. coli* and *S. aureus*. 

A research conducted by Nofiani et al. (2009) stated that extract methanol of bacteria associated with sponges showed antibacterial activity but based on minimum inhibition concentration value, antimicrobe activity still categorized as weak. Both extract fractions of n-hexane and ethyl acetate of isolate A2 did not show presence of antibacterial activity. This might due to the presence of other secondary metabolites that associated in each other and this indication has not detected thus capable of producing antibacterial activity against bacteria test. Other factors were the amount of secondary metabolite fraction obtained was small thus it was not capable of inhibiting the bacteria test growth.

### 3.8 Chemical Compound Assay of Extract Fraction n-Hexana, Ethyl Acetate and Methanol of Potential Bacterial Isolate

Based on screening results of antibacterial activity extract fraction of EH2F3, EA1F3, MA2F2, and MA2F3 have shown content saponin and alkaloid against bacteria test. Furthermore, test of chemical compound for secondary metabolite was conducted. It showed that MA2F2 and MA2F3 were positive contained saponin while EH2F3 and EA1F3 was positive contained alkaloid. Saponin for MA2F2 and MA2F3 and alkaloid for EH2F3 and EA1F3, have the same result with previous test of secondary metabolite compound. Secondary metabolite such as alkaloid and saponin are potential as antibacterial. According to Zheng (2005), secondary metabolite alkaloid of bacteria associated with sponges *Himeniacidon parleve* also showed antibacterial activity against *S. aureus*. Antibacterial activity produced by symbiotic bacteria of sponges *Haliclona sp.*2 and *Axinellid sp.* are able to compete to occur in the sea ecosystem. According to Dash et al. (2009), symbiotic bacteria which produces antibacterial compound is able to form colony on the sponges surface. Saponin, tanin and flavonoid are secondary metabolites that have antibacterial activity. Saponin is bioactive compound to increase membran permeability thus cell hemolysis occur, when saponin interacts with bacterial cell, the bacterial cell will be broken or lysis. This could be made as opportunity to develop secondary metabolite for further research (Ganiswarna, 1995).

### Conclusions:

Based on research results, the conclusion of this research were as following:

1. Extracts of EH2, EA1, EA2, HA2 and MA2 have antibacterial activity on bacteria *S. Aureus*, *E. Coli* and *P. Aeruginosa*.
2. Extract of EH2 was very potential against *S. aureus* and *E. Coli* compared with extracts EH2, EA1, HA2 and MA2.
3. It was found 4 (four) extract fractions of secondary metabolites (EH2F3, EA1F3, MA2F2 and MA2F3) which have an activity against S. aureus, E. coli and P. aeruginosa.

4. Based on phytochemical screening of secondary metabolite fractions (EH2F3, EA1F3, MA2F2 and MA2F3) that has antibacterial activity were alkaloid and saponin.

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