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Antibacterial And Antifungal Activity Of Sponge Extract From Natuna Water, Riau Islands.

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ABSTRACT

A Sponge is one of the marine animals that contains a compound that is potentially developed as a medicinal ingredient, one of them as antibacterial and antifungal. This research aims to determine the potential antibacterial and antifungal of sponge derived from the sea waters of Natuna, Riau Islands. The microbial test used is *Staphylococcus aureus* and *Candida albicans*. Testing antibacterial and antifungal activity using the diffusion method is done by measuring the diameter of the barrier around the paper disc, using various concentrations of 20%, 40%, 60%, 80%. The results showed that Natuna sea sponge extract was able to inhibit the growth of *Staphylococcus aureus* bacteria with the formation of an average inhibitory diameter at each concentration of 15.8 mm; 16.26 mm; 17.9 mm; 20.7 mm. Next to the fungus *Candida albicans* formed an average barrier diameter of 13.1 mm; 17.23 mm; 20.23 mm; 23.1 mm. Natuna Sea Sponge Extract has a very strong potency to be developed as an antibacterial and antifungal.

Keywords: Antibacterial, Antifungal, Natuna, Sponge.

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INTRODUCTION

Indonesia, especially the Riau archipelago has abundant wealth of marine biodiversity. Until now, it is used only fish as food products, but still many other marine biota potentially as medicine. One of them is sponge which is one of the invertebrate animals that has a biological activity that is useful in the treatment of an illness, one of which as antibacterial and antifungal [1, 2, 3].

Infectious diseases caused by fungi and bacteria are still very high and fewer antifungal drugs than antibacterial, while many antibacterial drugs have undergone resistance [4.5]. Therefore, it is necessary to develop antibacterial and antifungal drugs that come from marine biota, such as sponge.

Sponge is one of the invertebrates of the Porifera phylum which produces an active compound with a variety of structures and one of its biological activities is as an antimicrobial [6; 7.8]. Research conducted by [9] in 2012 about the isolation of the bioactive sponge compounds *Callyspongia* sp. with thin-layer chromatography (TLC) method is obtained that the sponge has a chemical content of alkaloids, flavonoids, and terpenoids. The results of this study are also supported by previous research, that sponge contains alkaloid compounds, flavonoids, steroids and antrakuinon [10].

Various active substances have been successfully isolated and identified from Indonesian sponges, such as Barangamide, Brianthein, Aaptamin, Lembehyne and Bitungolides [11]. Vilas et al., (2015) has proved that Aeroplysinin in the sponge *Ianthella Ardis* and *Aplysina Aerophoba* have strong antibiotic and anti-inflammatory effects on Gram-positive bacteria [12]. Ismet et al., (2016) has also researched 7 sponge which have pontension as an antibacterial against *Escherichia coli* and *Stapylococcus aureus* namely Clathria sp., Iotrochota sp., Sprastela sp, Agelasidae, Haliclona spp., Aplysina Aeorophoba, and Agelas conifer [13]. Other studies have also reported that the sea sponge extract Callyspongia sp can inhibit the growth of *Staphylococus aureus* bacteria [14].

Other compounds are also reported to have antimicrobial activity such as Caminoside A, a glycolipid isolated from the Camininphaeroconia species, 1.2-dioxane ring peroxide acid of Familia Plakiniidae and Swinhoeiamide A, a derivative calyculin of the sponge *Theonella Swinhoei* which is also reported active against pathogenic fungi of *Candida albicans* and *Aspergillus fumigatus* with KHM respectively 1.2 and 1.0 mg/mL [15.16]. The research has been done by Astuti et al. (2003) against 15 sponge extracts collected from Bunaken Marine Park, Belita Bay and Barrang Lompo Island and all extracts from the Bunaken Marine park are active against the bacteria of S. *aureus*, S. *Typhii*, and E. *coli* as well as C. *albicans* in concentrations of 1000 mg/ml. This makes sponge one of the most attractive marine animals to be researched and developed as antifungal and antibacterial [17].

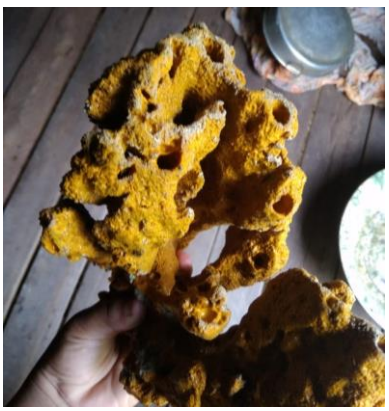

Figure 1: Sponge taken in Natuna waters

Based on the study of antibacterial and antifungal activity of sponge, there is no report of the pharmacological activity of sponge originating from Natuna water, Riau Islands (Figure 1). So the authors are interested to examine the antibacterial and antifungal activity of the sea sponge extracted from the waters of Natuna, so it can be utilized as an infectious disease drug.

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RESEARCH METHODS

Tools and Materials

Tools

Tools used by Digital scales (Kenko), Beaker glass (Pyrex Iwaki), Erlenmeyer (Pyrex Iwaki), measuring glasses (Pyrex Iwaki), glass bottles of chocolate, rotary evaporator (Heidolph made in Germany), Volume pipette (Pyrex), pipette drops, mouthpiece (Pyrex Iwaki), Reaction tube (Pyrex Iwaki), tweezers, Micro pipettes 50 µl, stirrer rod, Petri dish (Normax), needle ose, Incubator (Memmert), disc paper, ruler, autoclave, LAF (laminar air Flow) (Magnehelic) , hot plates (Maspion S. 302), water shaker, magnetic stirrer, long-term.

Material

Marine biota (sea sponge), methanol, aquadest, pure bacterial culture Staphylococcus aureus, fungus Candida albicans, HCl 2 N, Pereaksi Mayer, HCl concentrated, $fecl_3$ 1%, NaCl 0.9%, Dimetilsulfoksida (DMSO), Nutrient Agar (NA) (Merck), MHA (Mueller Hinton Agar), chloramphenicol, ketoconazole, H_2SO_4, $Bacl_2$. $2H_2O$, disc paper, aluminium foil.

Research Procedure

Sampling

Samples taken on February 7, 2020, in Natuna Water, Kelarik village, North District, Natuna Island Riau Islands at a depth of ± 15 meters below sea level. Then the sponge is cleaned using sea water and running water. Samples were immediately inserted into the bottle and soaked with methanol.

Sample Extraction

A fresh sample of sponge 4 kg that has been washed, Ditiriskan, and then macerated with methanol to be completely submerged in chocolate glass bottles and stored in a light protected place for 3x5 days while occasionally stirring and filtered with filter paper. The Maserat methanol is combined with a Rotary Evaporator until a condensed extract is then weighed 99.74 grams [18.19].

Media creation

A total of 5 grams of nutrient to be dissolved with 250 mL of the aquadest in the Erlemeyer and heated over the hot plate using a stirring stem until a clear solution is formed. Then sterilised in autoclaved at a temperature of 121 ° C pressure 2 atm for 15 minute. Nutrient to be inserted into several reaction tubes with a predetermined amount, the tubes that have been filled to be placed on the slope of 30-45o. Allowed to be cold and harsh [30].

Bacterial rejuvenation

Taken one colony of each of the Staphylococus aureus bacteria and Candida albicans by using a sterile OSE needle was then implanted in the media to be skewed by the way it was incubated after it was incubated at the incubator at the temperature of 37oC for 24 hours [31].

Sample investigation

The parent solution is made in the initial way of weighing 4 gr exfoliated, then dissolved in 5 ml dimethylsulfoxide (DMSO). From the 80% parent solution was then made dilution 20%, 40%, 60%. The negative control used (DMSO) 10 µl and the positive control used for antibacterial is Chloramphenicol 10%, the positive control used for the Antifungi is Chloramphenicol [22].

Manufacture of Mc. Farland solution

Solution H_2SO_4 1% as much as 99.5 ml mixed with BaCl solution $_2$. $2H_2O$ 1.175% as much as 0.5 ml in erlenmayer. Then whipped until formed murky solution. This turbidity is used as a test of turbidity of testing bacteria [32].

Bacterial suspension Manufacturing

The 24-hour-old bacterial biakan is taken from the sloping 2 OSE bacterial colony test resuspened into 10 ml NaCl 0.9% sterile in a sterile reaction tube. It is then homogenized with a vortex. Turbidity compared to MC Farland [32].

Fungal suspension making

The mushroom is taken 2 ose of the mushroom colony of Candida albicans aged 24 hours and then resuspensed into 10 ml of the aquadest in a sterile reaction tube.

Testing antibacterial activity

A total of 30 ml of nutrient agar (NA) is inserted into a sterile petri dish, then added 50.0 µl bacterial suspension. It is then homogenized by means of the wiggle petri dish containing the media until it meets all media surfaces. The Media is then compacted. Sterile discs soaked in each of the test solution of marine sponge extracts with a concentration of 20%, 40%, 60%, 80. The disc is attached to the surface. As negative control is used DMSO 10 ml and positive control is used Chloramphenicol 10%. This treatment is then repeated 3 times. Then the petri dish was incubated in the incubator for 18-24 hours at a temperature of 37 ^{o} C. Then the antibacterial activity is determined by measuring the diameter of the barrier zone formed by using a wheeled term [33].

Antifungal activity Testing

The suspension of the fungus Candida albicans is 100 µl inserted into a sterile petri dish, then inserted the MHA medium which is still liquid as much as 10 ml, and the media in the Allow to compress. Then on the medium of MHA is placed sterile disc paper that has been soaked with a thick extract of marine sponge with a concentration of 20%, 40%, 60%, 80%. The disc paper is placed on the surface of the media using a tweezers and pressed slightly. Then the petri dish incubated for 3-5 days at a temperature of 25-27 ^{o} C. The positive control used is Ketokenazol and the negative control is DMSO. After 72 hours observed there was no clear zone around the disc paper.

Data Analysis

Data analysis is performed descriptively in the form of tables by observing the measurement of the diameter of the barrier zone of the clear-enamelled region of the sea sponge extract [34].

RESULTS AND DISCUSSION

Test the marine sponge activity begins with the sampling of sea sponges in the area of Kelarik village, district of North Humuran, Natuna Island Riau Islands at a depth of ± 15 meters below sea level. Then the sponge is cleaned using sea water and running water. After being able to get a sample with a description of the branches trunk is brown, soft texture and smell fishy like fish. A sample of the research was shown in Figure 1. The samples were then watered with methanol to reduce decay, and then finely cut to the extraction process immediately [18].

The method used for extraction is maceration by soaking the sample with methanol for 3-5 days. Every 24 hours Filtratnya is filtered and the paper is macerated again with methanol. The solvents used are methanol because this solvent is a universal solvent that can dissolve all organic compounds, both polar and non-polar with a low boiling point (67 °c), making it easy to darken [18.19].

All methanol extracts are obtained, evaporated in vacuo in vacuum using a Rotary evaporator. Of the 4 kg wet sample of Natuna Sea sponge extract is obtained as many as 99.74 grams. Once obtained condensed extracts, performed phytochemical screening. From the identification it is obtained that the marine sponge extract contains alkaloids, flavonoids, terpenoids and saponins. It is almost the same as previous research on the sea sponge of the Mandeh Sumbar Islands [18.19].

Continued to antibacterial test using the Gram-positive bacteria *Staphylococus aureus* and antifungal using the fungus Candida albicans. These bacteria and fungi are widely found as food contaminants sold unhygienic so that it can cause gastrointestinal diseases, such as diarrhea [20].

Testing of antibacterial and antijmur activity is done by the diffusion method of disc paper. The diffusion method was chosen because this method has the simplicity, ease of antibacterial tests, and the ability to more easily inhibit the growth of bacteria and fungi [21.22]. The Diameter of this bacterial growth is characterized by the presence of a clear zone around the disc, forming a clear zone around the disc caused because of the area of bacterial growth inhibited by test samples [23].

The solvent used for dilution of the extract is DMSO. DMSO solvents have the advantage of being able to dissolve both polar and non polar compounds, in addition to being used as a solvent, DMSO is also used as a negative control. According to Jacob & Wood (1967) DMSO is reportedly relatively no effect on cell proliferation so as not to interfere with the observation results testing of antibacterial activity with diffusion method for [24, 21,22].

Chloramphenicol is chosen as a positive control of bacteria because it is widely spectrum for effective gram-positive and gram-negative bacteria. It is easily soluble in fat so that it penetrates bacterial cells [25, 26,27]. While as a positive control of the fungus used antibiotic ketoconazole which is a compound of imidazole, antispools activities by means of causing the irregularities of the membrane cytoplasm of fungi and affects the biosynthesis of ergosterol in cell fungi [26.27].

The results of the test antibacterial activity of the Natuna sponge extract against the Staphylococus aureus bacteria can be seen in table 1.

Table 1: Test result antibacterial activity sponge extract of Natuna water against the Staphylococus aureus bacteria

Sample	Concentration (%)	The Diameter of the Power (mm)			Average (mm)
		I	II	III	
Sea Sponge Extract	20	15,1	16,1	16,1	15,8
	40	16,1	16,1	16,6	16,26
	60	16,1	19,6	18,1	17,93
	80	19,3	21,4	21,4	20,7
Control + Chloramphenicol	10	32,6	32,6	32,6	32,6
Control – (DMSO)	-	-	-	-	-

From table 1, it can be seen that the average diameter of the sea sponge extract of Natuna at a concentration of 20%, 40%, 60%, 80% of the *Staphylococus aureus* bacteria are 15.8 mm, 16.26 mm, 17.93 mm, and 20, 7 mm while the positive control of chloramphenicol is 32.6 mm. This shows that the diameter of Natuna's sea sponge is almost closer to the diameter of the positives control resistance , sponge extract means potentially antibacterial. The form of a formed obstacle zone can be seen in Figure 2.

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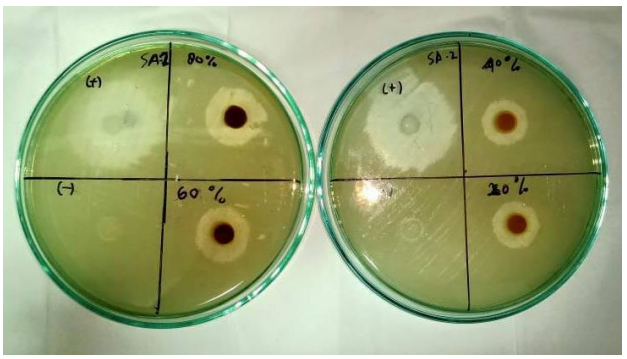

Figure 2: An obstacle zone formed against the Staphylococus aureus bacteria

While the test result of antifungal activity of Natuna sponge extract against the fungus *Candida albicans* can be seen in Table 2.

Table 2: Test result antibacterial activity sponge extract of Natuna water against the fungus Candida albicans

Sample	Concentration (%)	The Diameter of the Power (mm)			Average (mm)
		I	II	III	
Sea Sponge Extract	20	15,1	13,1	11,1	13,1
	40	17,1	15,5	19,1	17,23
	60	20,1	21,1	19,5	20,23
	80	25,1	22,1	22,1	23,1
Control + Ketokenazole	10	32,1	32,1	32,1	32,1
Control – (DMSO)	-	-	-	-	-

From table 1, it can be seen that the average of the diameter of the sea sponge extract of Natuna at a concentration of 20%, 40%, 60%, 80% against the fungus *Candida albicans* was 13.1 mm, 17.23 mm, 20.23 mm, and 23.1 while the positive control of ketokenazol amounted to 32.1 mm. This means that the sea sponge extract taken from Natuna The form of an obstacle zone that occurs in the fungus *Candida albicans* can be seen in Figure 3.

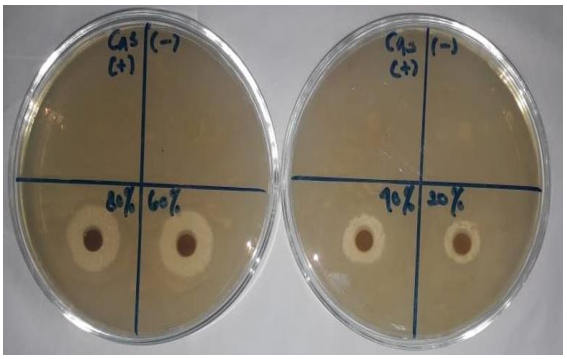

Figure 3: An obstacle zone formed against the fungus Candida albicans

Based on the results of observations conducted in the resistance test, indicating that with the administration of sponge extracts on the NA media that has been inoculated with bacteria and fungal test obtained the barrier zone, meaning the sea sponge extract of Natuna water has antibacterial and antifungal effect. This is in line with previous research related to sponges taken in other areas. The wide diameter of the hating zone formed around the disc can be used as a parameter to see the strength of the bioactive compounds

contained in the sea sponge. The wider the clear zone formed, the stronger the bioactive compounds that inhibit microbial growth [28].

The criteria of the hate zone strength as follows is a very strong category of barrier diameter ≥ 20 mm, strong 10-20 mm, medium 5-10 mm, and weak ≤ 5 mm [29]. Based on these criteria, the sea sponge that is taken in the waters of Natuna is very strong as both antifungal and as an antibacterial it is in line with the previous research on antibacterial and antifungal activities of sea sponges taken from various regions in Indonesia. It can be concluded that the higher the concentration of marine sponge extracts used then the stronger the antibacterial and antifungal power contained therein.

CONCLUSION

From the research that has been done can be concluded that Natuna sea sponge extract has antibacterial activity against *Staphylococus aureus* bacteria and has antifungal activity against the fungus *Candida albicans*. The higher the concentration of sponge extract then the greater in inhibiting the growth of microbial tests where the most inhibitory power is at concentrations of 80% and is classified as a very strong antibacterial or antifungal.

REFERENCES

[1] Ngantung, A., Bara, R., & Sumilat, D. (2017). Test the antibacterial activity of the Dictyonella Funicularis sponge and Phyllospongia Lamellosa taken on the waters of Bunaken. Coastal and tropical Sea journals, 4 (2), 10-16.

[2] Abubakar, H., Wahyudi, A. T., & Yuhana, M. (2011). Screening of bacteria associated with sponge Jaspis sp. As a manufacturer of antimicrobial compounds. Marine Sciences: Indonesian Journal of Marine Sciences, 16 (1), 35-40.

[3] Aristyawan, A. D. (2017). Antibacterial activity of ethanol extract from Sponge Agelas cavernosa (Doctoral dissertation, Airlangga University).

[4] Rosdianti, S., Dimara, L., & Ayer, P. I. L. (2019). Antifungal efficacy test of the sea star extract Linckia laevigata (Linnaeus, 1758) against the growth of Mushroom Trichophyton sp. ACROPORA: Journal of Maritime and Fisheries Sciences of Papua, 2 (2).

[5] Sukandar, D., S. Hermanto, and E. Lestari. 2008. Toxicity test of leaf extract of Pandan Wangi (Pandanus Amaryllifolius Roxb.) with Brine Shrimp Lethality Test (BSLT). J. Valensi. 1 (2): 63 – 70.

[6] Yulianty, R., Rante, H., Alam, G., & Tahir, A. (2011). Screening and analysis of KLT-biographies of antimicrobial compounds of several extracts from the sea waters of Barrang Lompo Island, South Sulawesi. Journal of traditional Medicine magazines, 16 (2), 88-94.

[7] Saroinsong, M. S., Kandou, F. E., Papu, A., & Singkoh, M. F. (2014). Test the power of methanol extracts of several types of Porifera against Escherichia coli and Staphylococcus aureus bacteria. MIPA Journal, 3 (2), 129-133.

[8] Hafizah, I., Muliati, F. F., & Sulastrianah, S. (2017). Antibacterial activity of ethanol extract Porifera (Spongia Officinalis) against Staphylococcus Aureus ATCC 25923. MEDULA, 4 (1).

[9] Krisyuninda, P. M. (2012). The toxicity test is a sponge fraction Callyspongia sp. With the brine shrimp test (BST) from the white sand waters of Situbondo. Surabaya: The tenth November Institute of Technology.

[10] Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical microbiology reviews, 12(4), 564-582.

[11] Rachmaniar, R. (2003). Antikanker Swinholide A dari Spons Theonella swinhoei. Jurnal Bahan Alam Indonesia, 2(4), 122.

[12] Vilas, J. A. G, Poveda, B. M., Quesada, A. R. & Medina, M. A. (2015). Aeroplysinin -1, a sponge derived multi targeted bioaktive marine drug. Jurnal of marine drug, 14, (1), 2-12.

[13] Ismet, M. S., Bengen, D. G., Radjasa, O. K., & Kawaroe, M. Composition and activity of sea Sponges from seagrass ecosystem in Thousand Islands waters, Jakarta Composition And Antibacterial Activities Of Marine Sponges From Different Seagrass Ecosystems In thousand Waters Islands, Jakarta.

[14] Warbung, Y. Y. (2013). The power of sea sponge extract Callyspongia sp against Staphylococcus aureus bacterial organization. e-GiGi, 1(2).

[15] Linington, R. G., Robertson, M., Gauthier, A., Finlay, B. B., van Soest, R., & Andersen, R. J. (2002).

Caminoside A, an antimicrobial glycolipid isolated from the marine sponge Caminus sphaeroconia. Organic letters, 4(23), 4089-4092.

[16] Chen, Y., McCarthy, P. J., Harmody, D. K., Schimoler-O'Rourke, R., Chilson, K., Selitrennikoff, C., ... & Wright, A. E. (2002). New bioactive peroxides from marine sponges of the family plakiniidae. Journal of natural products, 65(10), 1509-1512.

[17] Astuti, P., Alam, G., Pratiwi, S., Hertiani, T., & Wahyuono, S. (2019). Screening of the anti-infectious sponge compound from Bunaken, Manado. BIOTA: scientific Journal of Life Sciences, 8(2), 47-52.

[18] Handayani, D., Handayani, C., & Krisyanella, K. (2016). The main chemical compounds isolation from the cytotoxic active fraction of the Petroxia sea Spons sp (MN05). Jurnal Farmasi Higea, 4(1), 24-30.

[19] Krisyanella, H. D. (2011). Yunance L. Test the cytotoxic activity of extracts and fraction of the Petrosia SP's Marine sponge with the brine shrimp lethality test method. Jurnal Sains dan Teknologi Farmasi, 16(2), 156-166.

[20] Tan, S. L., Lee, H. Y., & Mahyudin, N. A. (2014). Antimicrobial resistance of Escherichia coli and Staphylococcus aureus isolated from food handler's hands. Food Control, 44, 203-207.

[21] Biemer, J. J. (1973). Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. Annals of Clinical & Laboratory Science, 3(2), 135-140.

[22] Bakhtra, D. D., Fajrina, A., & Irenda, Y. (2018). Assay activity of Ektrak Etil acetate sponge Aplysina aerophoba on Helicobacter pylori and Shigella dysenteriae. Jurnal Farmasi Higea, 10(2), 134-142.

[23] Perilla, M. J. (2003). Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world; Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Neisseria gonorrhoeae, Salmonella serotype Typhi, Shigella, and Vibrio cholerae.

[24] Jacob, S. W., & Wood, D. C. (1967). Dimethyl sulfoxide (DMSO) toxicology, pharmacology, and clinical experience. The American Journal of Surgery, 114(3), 414-426.

[25] Siswandono & Soekardjo, B. (2000). Medisinal chemistry. (II edition). Surabaya: University of London.

[26] Peristiwaningrum, A. (2019). Antibacterial activity screening of methanol extract some sponge from the island of Banyuwangi taapse against Bacillus subtilis and Pseudomonas aeruginosa (Doctoral dissertation, Universitas Airlangga).

[27] Yulianty, R., Rante, H., Alam, G., & Tahir, A. (2011). Screening and analysis of KLT-biographies of antimicrobial compounds of several extracts from the sea waters of Barrang Lompo Island, South Sulawesi. Journal of traditional Medicine magazines, 16 (2), 88-94.

[28] Rumampuk, Y. B., Wowor, P. M., & Mambo, C. D. (2017). The Sea Sponge extract (Callyspongia Aerizusa) on the growth of Salmonella Typhi bacteria and Streptococcus Pyogenes. eBiomedik, 5(2).

[29] Davis, W. W., & Stout, T. R. (1971). Disc plate method of microbiological antibiotic assay: I. Factors influencing variability and error. Appl. Environ. Microbiol., 22(4), 659-665.

[30] Lay, B. W & Hastowo, S. (1994). Laboratory microbial analysis. (edisi 1). Jakarta: PT Raja Grafindo Persada.

[31] Rusdi, N. K., & Sediarso, S. H. F. (2010). Antibacterial activity test of ethanol fraction 70% of God's Crown leaf extract (Phaleria macrocarpa (Scheff) Boerl.) To bacteria Stretococcus Streptococcus. Farmasains, 1, 89-94.

[32] Hardoim, C. C., & Costa, R. (2014). Microbial communities and bioactive compounds in marine sponges of the family Irciniidae—a review. Marine drugs, 12(10), 5089-5122.

[33] Misna, M., & Diana, K. (2016). Antibacterial activity of red onion skin extract (Allium cepa L.) against bacteria Staphylococcus aureus. Jurnal Farmasi Galenika (Galenika Journal of Pharmacy), 2(2), 138-144.

[34] Perdicaris, S., Vlachogianni, T., & Valavanidis, A. (2013). Bioactive natural substances from marine sponges: new developments and prospects for future pharmaceuticals. Nat. Prod. Chem. Res, 1(3), 2329-6836.