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1 ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT SPECIES SP2 AND SP4 WITH AGAINST STAPHYLOCOCCUS AUREUS, BACILLUS SUBTILIS, AND ESCHERICHIA COLI BACTERIA

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4 ABSTRACT

Utilization of marine biota as one of the raw materials in medicine becomes an interesting research material to be investigated where the magnitude of the potential bioactive compounds it contains. SP2 and SP4 soft coral samples taken in the Thousand Islands were extracted using ethanol, the extract was thickened and a thick extract was obtained which was then tested for its antibacterial activity. The antibacterial assay uses the diffusion method of wells and tested against the bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. The extract concentrations used were 15%, 25%, 35% and 45%. The positive control used was amoxicillin while the negative control used was CMC-Na. The results showed that SP2 soft coral extract was effective in inhibiting the growth of *S. aureus* bacteria with an average inhibition zone of 10.8 mm and included in the category of strong inhibition. As for SP4, it is effective in inhibiting the growth of *S. aureus* and *B. subtilis* bacteria with inhibition zones of 13.4 mm and 11.17 mm respectively, and is categorized in strong inhibition. Both soft coral extracts are effective in inhibiting bacteria from the gram-positive group due to the relatively simple nature of gram-positive bacterial cell walls making it easier for antibacterial compounds to enter the cell.

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INTRODUCTION

Soft coral is a marine biota that has begun to be developed into several pharmaceutical preparations. Various studies show that marine biota has enormous potential in producing active compounds that can be used as raw material for medicine. Soft corals have been widely studied and are known to have good antioxidant activity, due to their flavonoid content. Besides being known to have antioxidant activity, soft corals also have antibacterial, antifungal, cytotoxic, antineoplastic, HIV inhibitors and anti-inflammatory activities (Radhika, 2006). Previous studies have shown that *Xenia sp.* has antibacterial activity against the bacteria *Staphylococcus aureus* and *Escherichia coli* (Kantor et al., 2015). In another study where the *Nephtea sp.* species were tested, the ethanol fraction showed antimicrobial activity in *Escherichia coli* (Rumegan, 2013). In a study conducted by Huda et al., (2012) soft corals with *Sacrophyon sp.* species have antibacterial activity against *Staphylococcus aureus*. Soft coral resources in the Thousand Islands are estimated to reach 103 species from 4 families (Manuputty, 1992). Of the amount, only a small part has been

studied its bioactive potential. On the other hand, soft coral understood as a reef biota that has bioactive materials from terpenoid compounds, which are cytotoxic, anticancer, algicidal, and antipredator. Thus, research activities and scientific studies of the types of soft corals in Indonesian waters known to have an area of coral reefs the widest in the world needs to be improved (UNEP, 2002). This research was conducted on several islands in the Thousand Islands, namely Pari Island, Pulau Scouts, and Kotok Island as locations for soft coral sampling. The objectives of the study are: (a) to determine the horizontal distribution and vertical soft corals that have bioactive potential in the Thousand Islands, DKI Jakarta, and (b) know the types of soft corals which has bioactive potential in the islands Seribu, DKI Jakarta.

Experimental Section

Extraction

Samples were extracted with ethanol solvent by the maceration method. Maceration is done for 24 hours with 3 repetitions. The result of extraction is a liquid extract. The liquid extract is

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then thickened with a rotary evaporator to remove the solvent contained in the liquid extract.

Phytochemical Screening

Alkaloid Test

As much as 4 ml of extract was put into a test tube then added 2 ml of chloroform and 5 ml of 10% ammonia, then added 10 drops of 2M sulfuric acid. Taken the top of the formed phase, then added Mayer. Positive results are indicated by the presence of red deposits (Harborne, 1996 in Rumagit, 2015).

Flavonoid Test

The extract was taken as much as 1 ml and then added with enough Mg powder and 10 drops of concentrated hydrochloric acid. Positive results are indicated by the presence of reddish-black, yellow, or orange.

Saponin Test

The test extract was added with 10 ml of hot water, then added 2N hydrochloric acid, and then shaken strongly. Formed stable froth for approximately 10 minutes after 1-10 cm.

Tanin Test

The extract was added with a solution of 10% iron (III) chloride. Positive results indicate dark blue, black, or greenish-black.

Steroid and Triterpenoid Test

Extract plus 5 ml of ether and pipetted. Put in the vaporizer cup until the solvent evaporates. The rate is added 2-3 drops of LB reagents. Positive results are indicated by the presence of a violet color (triterpenoid) and blue (steroid).

Bacterial Rejuvenation Test

Test bacteria in the form of *S.aureus*, *B.subtilis*, and *E.coli* derived from pure culture, taken one ose and then inoculated by etching in nutrient agar (NA) media then incubated at 370C for 24 hours. Then a bacterial suspension is made using NaCl as a growth medium.

Antibacterial Activity Test

The extract inhibition test was performed using the diffusion method of the wells. Prepared sterile nutrient media (NA) with a temperature of 45 -50⁰C as much as 15 ml, then poured aseptically into a sterile petri dish and allowed to solidify. Furthermore, the bacterial suspension that has been prepared, rubbed into the agar media. Next, perforate the agar media with wells and each hole filled with extracts with concentrations of 15%, 25%, 35%, and 45% for sp2 and sp4. The extract was suspended in 1% CMC Na. Furthermore, it was incubated at 370C for 24 hours. The positive control used was Amoxicillin and the negative control used was CMC-Na.

Data analysis

Data analysis was performed using SPSS Statistics 20. The normality test was performed by the Shapiro Wilk method and then followed by a homogeneity test with the Levene Test. Follow-up tests use ANOVA if the data obtained are normally distributed and have the same data variance if the data are not normally distributed and are not homogeneous, the test is

performed using Kruskal Wallis followed by the Mann-Whitney test.

RESULTS AND DISCUSSION

Soft Coral Extraction

Soft coral extraction was carried out using the maceration method with ethanol as a solvent. The choice of ethanol as a solvent is due to its polar nature so that it can attract polar to non-polar compounds. The choice of the maceration method as an extraction method is due to the active compounds in soft corals that have antibacterial properties as flavonoids. According to Koirewoa et al. (2012) and Rompas et al., (2012), flavonoid compounds are heat-resistant compounds, besides flavonoid compounds are easily oxidized at high temperatures.

Table 1 Extract

Sampel	Weight of simplisia (gram)	Concentration Results (gram)	% Yield
SP2	250	15,54	6,2%
SP4	250	7,48	3,0%

Phytochemical Screening

Based on the results of phytochemical screening, it was found that the ethanol extract of SP4 and SP2 soft corals positively contained secondary metabolites of flavonoid. The mechanism of action of flavonoids as an antibacterial is that it inhibits the function of cell membranes by forming complex bonds with cell walls and damaging membranes. Also, it inhibits energy metabolism by inhibiting the respiratory system, because it requires sufficient energy for the active absorption of various metabolites and biosynthesis of macromolecules (Cushinie, 2005 in Nuraina).

Antibacterial Activity Test

The antibacterial activity test of SP2 and SP4 soft coral extracts was carried out on three bacteria, namely *Staphylococcus aureus*, *Bacillus subtilis*, which were bacteria from the gram-positive group and *Escherichia coli* from the gram-negative group. The method used is a diffusion well or Cup-plate technique with concentrations of 15%, 25%, 35%, and 45% for SP2 and SP4 extracts. CMC-Na is used as a negative control because it is known to have no antibacterial activity. A negative control is also used as extract thinner with the aim that negative control does not affect the extract activity test (Natheer, 2012). Amoxicillin 1% is used as a positive control because it has a broad-spectrum activity that is effective against both gram-positive and gram-negative bacteria.

Table 2 Antibacterial activity test results of SP4 soft coral ethanol extract

Concentration(%)	Inhibition zone		
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>E. coli</i>
15	7,5 mm ± 0,89 ^a	5,63 mm ± 1,30 ^a	7,2 mm ± 1,40
25	8,4 mm ± 1,29 ^b	9,2 mm ± 1,04 ^{ab}	7,9 mm ± 1,10
35	9,4 mm ± 1,51	9,9mm ± 1,28	8,0 mm ± 1,20
45	13,4 mm ± 2,83 ^{abc}	11,17 mm ± 1,18	8,4 mm ± 0,80
Positive-controll	20,67 mm ± 3,21 ^c	21,53 mm ± 4,65 ^b *	22,2 mm ± 3,47

Note: a-c = number followed by the same letter shows a significant difference (p < 0.05)

Table 3 Hasil uji aktivitas anti bakteri ekstrak etanolka ranglunakS2

Concentration (%)	Inhibition zone		
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>E.coli</i>
15	7,7 mm ± 0,00 ^{ac}	0,0 mm ± 0,00 ^{ac}	0,0 mm ± 0,00
25	0,0 mm ± 0,00 ^{bc}	0,0 mm ± 0,00 ^{bc}	0,0 mm ± 0,00
35	6,7 mm ± 1,20 ^{ab}	8,7 mm ± 2,10 ^{ab}	0,0 mm ± 0,00
45	10,8 mm ± 3,10 ^{ab}	10,1 mm ± 1,90 ^{ab}	0,0 mm ± 0,00
Positive-Controll	26,67 mm ± 9,29 ^c	23,43 mm ± 2,98 ^c	25,03 mm ± 4,54

Note: a-c = number followed by the same letter shows a significant difference (p < 0.05)

Inhibition zone diameter less than 5 mm indicates inhibitory activity in the weak category, diameter 6-10 mm is categorized as having moderate activity, diameter 11-20 mm is categorized as having a strong activity and diameter of inhibition zone is more than equal to 21 then categorized as having a very strong activity (Susanto *et al.*, 2012). Based on the results of research that has been done, SP2 extract has antibacterial activity against *S. aureus* with moderate inhibition category at a concentration of 35% with an average zone diameter of 6.7 mm inhibition. As well as a strong category for 45% concentration with an average inhibition zone diameter of 10.8 mm. The results can be seen in Figure 4.1. For inhibitory activity in *B. subtilis* bacteria, SP2 extract with a concentration of 35% has moderate activity with an average zone diameter of 8.7 mm, and for a concentration of 45% has strong activity with an average inhibition zone diameter of 10.1 mm. The results can be seen in Figure 4.2. Meanwhile, which has no inhibitory activity on the growth of *E. coli* bacteria. For SP4 extract, it has moderate activity at concentrations of 15%, 25%, and 35% with an average inhibition zone diameter of 6.5 mm; 7.5 mm and 8.13 mm against *S. aureus* bacteria. Has a strong activity category at a concentration of 45% with an average inhibition zone diameter of 10.1 mm. The results can be seen in Figure 4.3. In *B. subtilis* bacteria, SP4 extract had moderate activity at a concentration of 15%, 25%, and 25% with each inhibition zone diameter of 5.63 mm each; 9.2 mm and 9.9 mm and strong category for 45% concentration with inhibition zone diameter of 11.17 mm. The results seen in Figure 4.4 in *E. coli* bacteria have activity in the medium category for concentrations of 15%, 25%, 35%, and 45% with each inhibition zone diameter of 7.2 mm; 7.9 mm; 8.0 mm and 8.4 mm.

From the above results, SP4 and SP2 soft coral ethanol extracts are effective in inhibiting the growth of *S. aureus* and *B. subtilis* bacteria which are a group of gram-positive bacteria. The ability of extracts to inhibit bacterial growth is influenced by the cell wall properties of the test bacteria (Maradona, 2013). Gram-positive bacteria have a simpler cell wall structure making it easier for antibacterial compounds to enter the cell and reach its workplace while gram-negative bacteria have better resistance than gram-positive bacteria against antibacterial compounds (Maradona, 2013). In its structure, the cell wall of gram-positive bacteria consists of 90% peptidoglycan while the gram-negative bacterial cell wall layer contains only 5-10% of the remaining peptidoglycan which is protein, lipopolysaccharide, and lipoprotein (Madigan *et al.*, 2005). Gram-positive bacteria have 3-5 times greater osmotic pressure compared to gram-positive bacteria so that they are easily lysed (Maradona 2013). Without cell walls, bacteria can

not stand the influence of the surrounding environment and will soon die.

In a study conducted by Fatmawati (2015) who tested the antibacterial activity of soft corals with the species *Sarcophyton* against *S. aureus* bacteria, the results showed that at a concentration of 15% showed a strong category with inhibition zone diameter of 11.18 mm and included in the medium category at concentrations of 10% and 5%. In other studies, the same species was tested for ethyl acetate extracts against the bacteria *S. aureus*, *E. coli*, *B. cereus*, and *P. aeruginosa* showing activity in the weak category (Hardiningtyas, 2009).

In another study, soft corals with species of *Nephtea* ethanol fraction tested on *E. coli* bacteria showed antibacterial activity concentrations of 1 mg/ml with inhibition zones of 0.88 mm and were categorized as having no activity against *E. coli* bacteria (Rumengan, 2013).

CONCLUSION

SP2 soft coral ethanol extract was most effective in inhibiting the growth of *S. aureus* bacteria at a concentration of 45% with a diameter of inhibitory zone of 10.8 mm and included in the category of strong inhibition, in *B. subtilis* bacteria with a concentration of 45% had an inhibitory zone diameter value of 10.1 mm and falls into the medium inhibition category. And does not show antibacterial activity in the *E. coli* bacteria group.

The SP4 soft coral ethanol extract was the most effective in inhibiting the growth of *S. aureus* and *B. subtilis* bacteria are seen from the inhibition zone diameter with values of 13.4 mm and 11.17 mm and categorized in the strong inhibitory category. Whereas, *E. coli* bacteria had an inhibition zone diameter value of 8.4 mm and included in the category of moderate inhibition.

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