# Antimicrobial Activity of Marine Bacteria Extract from The Coastal Area of Serdang Bedagai North Sumatera

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**ABSTRACT**: Screening of marine bacteria shown thirty six isolates had inhibition zone around colony. Three potential isolates SB5, BG3 and BG4 extracted with methanol, ethyl acetate extract and n-hexane. Ethyl acetate extract had antimicrobial activity againts *Staphylococcus aureus, Eschericia coli, Bacillus subtilus,* while, n-hexane extract did not show antimicrobial activity againts *S. aureus, E. coli, B. Subtilus.* Antimicrobial activity againts *C. albicans* was showed by methanol extract of BG4, ethyl acetate extract of SB5 and BG3, and n-hexane of SB5 at high concentration. Ethyl acetate extract of BG4 and methanol, ethyl acetate, n-hexane extract of SB5 showed antimicrobial activity againts *Vibrio sp*. antimicrobial activity SB5 extracted ethyl acetate had antimicrobial activity againts gram positive bacteria, gram negative bacteria and yeast.

**KEYWORDS**: marine bacteria extract, antimicrobial activity, inhibition zone

## 1. INTRODUCTION

The marine environment has a different microorganisms that are widespread and not owned by the terrestrial environment. Based on the research results of the National Research Council (2002)[1], only 1% of total marine bacteria that can be cultured by a commonly used method. This means 99% of the products of chemical compounds from marine microorganisms have not been studied and has the potential to be applied commercially. The development of methods of cultivation and detection is an attractive strategy to increase the percentage of bacteria that can be cultured (Zengler et al., 2002)[2].

Early stage to obtain the active compounds from marine organisms is to perform screening. The next step after the screening process is the purification and identification of components that can indicate biological activity was detected in extracts containing the active components (Pelaez, 2005)[3]. Bioactive components of the sea can be applied in various industries, namely: medical, pharmaceutical, cosmetics, food supplements, chemicals derived from organisms, microorganisms, and associated organisms-organisms (Mahyer & Lehman, 2001)[4].

Serdang Bedagai has the potential of marine sizeable geographical position directly opposite the Strait of Malacca, which is access to fishing in the exclusive economic zone and the high seas. A coastline of 95 km covering five districts, namely: Pantai Cermin, Perbaungan, Noni bay, Tanjung Beringin and Bandar Khalifah. Marine potentials not only in the fisheries sector, but can also be developed from the use of organisms or microorganisms found in deep sea habitats produce bioactive components.

## 2. RESEARCH METHODS

### *Screening of bacteria producing antimicrobial from seawater samples*

Isolation of bacteria were performed on samples of sea water taken from local coastal areas 5 Bedagai Serdang, North Sumatra, namely; Gudang Garam beach (N: 03039'27,3 ", E: 0980 58'25,6"), Kwala Princess beach (N: 03037'34,7 ", E: 0990 01'52,4"), Klang beach (N: 03034'43,7 ", E: 0990 06'31,8"), beehive Fruit beach (N: 03034'25,3 ", E: 0990 07'26,1"), Bogak beach (N: 03033 '24, 6 ", E: 099009'38,44").

Screening of bacteria carried by growing the bacteria on NA media with seawater. 0.1 ml samples of sea water caused by the media, then incubated for 24-96 hours at 30 ± 20C incubator. Potential antimicrobial owned isolates characterized by the formation of clear zones around colonies of bacteria (Bonev *et al*., 2008)[5]. From the 36 isolates potentially have three isolates for antimicrobial compound extraction process based on ability early in inhibiting microbes.

### *Antimicrobial extraction of Isolates Potential*

Bacteria that have antimicrobial activity inoculated on NA medium with sea water, incubated at room temperature for 5 days with a temperature of 300C ± 2. Then the solid medium chopped and macerated with methanol for 48 hours. Maceration re-done three times with the new solvent. The ethanol extract can be obtained by doing filtration and centrifugation at a speed of 1,000 x g for 15 minutes. Supernatant is concentrated by rotary evaporator with a temperature of 500 C ± 2. The extract is

diluted with solvent DMSO accordance with a concentration of 25%, 50%, 75%. The same procedure is performed on solvent extraction using ethyl acetate and n-hexane.

***Antimicrobial Activity Testing Bacterial Extract***

Testing the antimicrobial activity of extracts consist of multiple treatments, namely: konsentraksi extract 100%, 75% concentration of the extract, the extract concentration of 50%, 25% extract concentration, and control by using the solvent DMSO. The method used is the disc diffusion method. Each extract was tested in microbial pathogen *S. aureus, E. coli, B. subtilis, C. albicans, Vibrio sp*. A total of 10µl extract concentrations determined dripped on the paper disc. Then the paper disc placed on the media in a petri dish containing the test isolates and incubated at a temperature of 300C ± 2 for 24 hours. Each treatment was carried out by 3 replications. Then measuring the inhibition zone formed by using a caliper in mm.

***Research design and data analysis***

The research design is done with a completely randomized design. Inhibition zone measurement data were analyzed using Analysis of Variance. If the results of the analysis will be significantly different from Test Duncan Multiple Range Test (DMRT).

## 3. RESULT AND DISCUSSIONS

The results of the initial screening phase obtained thirty-six isolates showed antimicrobial activity. There are 3 isolates SB5, BG3 and BG4 which have inhibitory zone large enough to test microbe *Staphylococcus aureus*, *Vibrio sp*., and *Candida albicans* respectively 31 mm; 27.9 mm; 26 mm. The existence of microbes in the growing medium is a competitor in acquiring nutrients and secrete antimicrobial compounds in response to defend itself and colonization of microbes. Important factors that play a role in the growth of bacteria isolated from water alaut among others; salinity, nutrient availability, antagonistic microbe interactions, and antibiotic substance (Hrenovic & Ivankovic, 2009)[6].

Statistical analysis of the test results show the ethyl acetate extract three potential isolates SB5, BG3 and BG4 has antimicrobial activity against *Staphylococcus aureus, E. coli* and *B. subtilis*. The results of the three races extraction using n-hexane showed no activity animikroba terhadap *B. subtilis*, *E. coli*., *S. aureus* (tables 1,2, and 3).

Table 1. Effect of Ekstract to The *B. subtilis*

| | Metanol | | | Etil Asetat | | | N-Heksan | | |
|---|---|---|---|---|---|---|---|---|---|
| Concentration | SB5 | BG3 | BG4 | SB5 | BG3 | BG4 | SB5 | BG3 | BG4 |
| 0 | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] |
| 25 | 0,71[a] | 0,71[a] | 0,71[a] | 3,33[b] | 3,33[b] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] |
| 50 | 0,71[a] | 0,71[a] | 3,68[e] | 3,36[b] | 3,36[b] | 3,36[b] | 0,71[a] | 0,71[a] | 0,71[a] |
| 70 | 0,71[a] | 0,71[a] | 3,69[e] | 3,52[d] | 3,54[d] | 3,45[c] | 0,71[a] | 0,71[a] | 0,71[a] |
| 100 | 0,71[a] | 0,71[a] | 3,71[e] | 3,71[e] | 3,71[e] | 3,54[d] | 0,71[a] | 0,71[a] | 0,71[a] |

******The figures in the table, followed by the same letter, no significant difference in $\rho \leq 0{,}05$. Figures followed by different letters, means significantly different at $\rho \leq 0{,}05$. Data is transformed by using the formula $\sqrt{x+\frac{1}{2}}$, x = the size of the clear zone

Extract BG3 and BG4 with methanol at a high concentration, each show can inhibit the activity of E. coli and S. aureus (Table 2 and 3).

Table 3. Effect of Ekstract to *S. aureus*

| Concentration | Metanol | | | Eti Asetat | | | N-Heksan | | |
|---|---|---|---|---|---|---|---|---|---|
| | SB5 | BG3 | BG4 | SB5 | BG3 | BG4 | SB5 | BG3 | BG4 |
| 0 | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] |
| 25 | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 3,35[b] | 0,71[a] | 0,71[a] | 0,71[a] |
| 50 | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 3,33[b] | 3,39[c] | 0,71[a] | 0,71[a] | 0,71[a] |
| 70 | 0,71[a] | 0,71[a] | 3,36[bc] | 3,38[c] | 3,37[bc] | 3,52[d] | 0,71[a] | 0,71[a] | 0,71[a] |
| 100 | 0,71[a] | 0,71[a] | 3,48[d] | 3,53[d] | 3,53[d] | 3,96[e] | 0,71[a] | 0,71[a] | 0,71[a] |

** The figures in the table, followed by the same letter, no significant difference in $\rho \leq 0{,}05$. Figures followed by different letters, means significantly different at ρ ≤0,05. $\rho \leq 0{,}05$. Data is transformed by using the formula $\sqrt{x+\frac{1}{2}}$, x = the size of the clear zone

The extract isolates with methanol BG4, BG3 extract and SB5 solvent ethyl acetate can inhibit the growth of *C. albicans.* In a large concentration of extract SB5 with n-hexane can also inhibit the growth of *C. albicans* (Table 4).

Table 4. Effect of Ekstract to *C. albicans*

| Concentration | Metanol | | | Eti Asetat | | | N-Heksan | | |
|---|---|---|---|---|---|---|---|---|---|
| | SB5 | BG3 | BG4 | SB5 | BG3 | BG4 | SB5 | BG3 | BG4 |
| 0 | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] |
| 25 | 0,71[a] | 0,71[a] | 3,33[b] | 3,33[b] | 3,34[bc] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] |
| 50 | 0,71[a] | 0,71[a] | 3,68[de] | 3,36[de] | 3,35[b] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] |
| 70 | 0,71[a] | 0,71[a] | 3,69[e] | 3,55[d] | 3,52[d] | 0,71[a] | 3,35[b] | 0,71[a] | 0,71[a] |
| 100 | 0,71[a] | 0,71[a] | 3,71[e] | 3,69[e] | 3,56[d] | 0,71[a] | 3,38[c] | 0,71[a] | 0,71[a] |

** The figures in the table, followed by the same letter, no significant difference in $\rho \leq 0{,}05$. Figures followed by different letters, means significantly different at $\rho \leq 0{,}05$. Figures followed by different letters, means significantly different at $\sqrt{x+\frac{1}{2}}$, x = the size of the clear zone

Table 5. Effect of Ekstract to *Vibrio sp*

| Concentration | Metanol | | | Eti Asetat | | | N-Heksan | | |
|---|---|---|---|---|---|---|---|---|---|
| | SB5 | BG3 | BG4 | SB5 | BG3 | BG4 | SB5 | BG3 | BG4 |
| 0 | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] | 0,71[a] |
| 25 | 0,71[a] | 0,71[a] | 0,71[a] | 3,33[b] | 0,71[a] | 3,35[d] | 0,71[a] | 0,71[a] | 0,71[a] |
| 50 | 3,38[d] | 0,71[a] | 0,71[a] | 3,38[d] | 0,71[a] | 3,39[e] | 3,25[b] | 0,71[a] | 0,71[a] |
| 70 | 3,53[e] | 0,71[a] | 0,71[a] | 3,53[e] | 0,71[a] | 3,5[e] | 3,35[d] | 0,71[a] | 0,71[a] |
| 100 | 3,71[f] | 0,71[a] | 0,71[a] | 3,71[f] | 0,71[a] | 3,85[g] | 3,38[d] | 0,71[a] | 0,71[a] |

**The figures in the table, followed by the same letter, no significant difference in $\rho \leq 0{,}05$. Figures followed by different letters, means significantly different at $\rho \leq 0{,}05$. Data is transformed by using the formula $\sqrt{x+\frac{1}{2}}$, x = the size of the clear zone.

Testing extract using a third solvent SB5 showed a fairly high antimicrobial activity against *Vibrio sp*. This is presumably because the compounds contained in the potential isolates dissolved well in all three types of solvents Isolate BG4 antimicrobial activity on *Vibrio sp*. from solvent extraction with ethyl acetate, the extract BG3 with a third solvent showed no antimicrobial activity (Table 5).

Differences in the effectiveness of the extract in inhibiting microbes occur due to differences in the composition of the active compounds are dissolved in a solvent third kind. The principle that the solubility of polar compounds dissolve polar, semi-polar solvent dissolves the compound semi-polar, non-polar solvents dissolve non compounds. Isolates with a third extraction solvent in some microbial tests also showed no antimicrobial activity is probably due to the gene encoding the formation of secondary metabolites are not expressed in normal circumstances that need to be induced by certain compounds.

Testing the antimicrobial activity of the extract in this study demonstrated the ability to inhibit the extract is more effective on gram-positive bacteria of the gram-negative. This is due to differences in the cell wall of gram-positive simpler than gram-negative bacteria, so that the extract can inhibit the growth and colonization of gram-positive bacteria. [7] Jayanth et al. (2001), showing gram-positive bacteria are more susceptible to the antibiotics produced by marine microorganisms and compared to gram-negative bacteria.

Good solvent used in this study is ethyl acetate, it's likely because ethyl acetate is a semi-polar can attract antimicrobial compounds contained in isolates with good potential and significant effect on the growth of microbes, *E. coli, B. subtilis, S . aureus, Vibrio sp., C. albicans.* Methanol is also a good solvent that can inhibit the growth of all microbes. Solvent n -heksana can only withdraw from isolates SB5 antimicrobial compounds, and can only inhibit the growth of *Vibrio sp., C. albicans* at high concentrations.

Testing extract isolates SB5 solvent ethyl acetate in microbial test showed broad-spectrum antimicrobial activity against gram-positive bacteria, gram-negative bacteria, and yeasts. From the research allegedly solvent ethyl acetate has good effectiveness in dissolving the active compound contained in isolates potential that can be secreted from the cell. Extraction with ethyl acetate demonstrated antibacterial activity with compounds produced pyrole, quinolinol, tyrosol, isatin and polysaccharides (Abraham, 2004)[8].

The results of this study have the same as that found on the exploration of marine bacteria in coastal Mangalore India. Isolates with ethyl acetate extracts showed activity out extensive sprektrum against gram-positive and gram-negative bacteria (Goklukrishan, 2011) [9]. Extraction isolates *Pseudoaltetromonas sp*. A1-J11 solvent ethyl acetate demonstrated the ability to inhibit the growth of *Vibrio sp.* (Castillo et al., 2008) [10]. [11] Isnanetyo and Kamay (2003), the methanol extract of marine bacterial culture *Pseudoaltetromonas phenolica* inhibits the growth of *E. coli* by changing the permeability of the membrane and attack the polysaccharide component on the outer membrane.

## 4. CONCLUSION

Based on the above results indicate extract treatment with different solvents and high concentrations capable of inhibiting the growth of microbes. This is due to the high concentration of activity is high antimicrobial compounds. Extraction with solvents of different polarity produces different bioactive compounds in accordance with the polarity of the solvent.

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