Phytochemical and Antibacterial Activity Test Of Secondary Metabolite Compound In *Rhizophora mucronata* Methanol Leaves Extracts

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Abstract—The purpose of this research are to phytochemical and antibacterial activity test of secondary metabolite compound in *Rhizophora mucronata* methanol leaf extract that obtained from Lappa village, Samataring District, East Sinjai Regency, South Sulawesi. This research was carried out in several steps, they were maceration, evaporation, phytochemical test and antibacterial activity. Phytochemical testing research results showed that the secondary metabolites contained in *Rhizophora mucronata* methanol leaf extract were flavonoids and steroids compounds. Antibacterial activity testing used *Staphylococcus aureus* and *Escherichia coli* bacterial was done with diameter stage resistor area (DDH) with 10%, 20%, 40%, 60% concentration. This research was conducted at the Laboratory of Chemistry and Biology, Mathamatic and Exact Faculty, Makassar State University. The test results showed that diameter stage resistor area of *Rhizophora mucronata* methanol leaf extract in 40% and 60% concentration most effectively inhibit the growth of *S. aureus* and *E. coli* bacterial because it has the IPD > 8 mm.

Keywords: Phytochemical, Antibacterial, R. mucronata, S. aureus, E. coli.

I. INTRODUCTION

Development of usage on natural ingredients as a traditional medicine to better use more in demand now. This is happened because traditional medicines are relatively easy to obtain and get supported by their ingredients from nature that grows abundantly in Indonesia. Usage of traditional medicine become more frequent and widespread in society. One of the alternatives that can be used is the active compounds of mangrove. Besides the abundant amount, mangroves have also been widely used as natural medicines. Some mangrove species have been used as a traditional and natural insecticide and pesticide. There are around 112 countries have mangrove and most of the area between 30° north and south of the equator and divided into 8 families and consists of 12 genera of flowering plants: *Avicennia, Sonneratia, Rhizophora, Bruguiera, Ceriops, Xylocarpus, Lummitzera, Laguncularia, Aegiceras, Aegiatilis, Snaeda*, and *Conocarpus* [8].

*Rhizophora mucronata* is one mangrove species that has antibacterial, antiviral and antifungal functions. An antibacterial substance that can inhibit or kill the bacterium causing the infection. Infections caused by bacteria or pathogenic microorganisms, which the microbes get into the body tissues and proliferate in the body networking. Among the bacteria that can cause infections are *Staphylococcus aureus* and *Escherica coli*. *Staphylococcus aureus* can cause pneumonia, meningitis, empyema, endocarditis or sepsis with suppuration in each organ [5].

Research on the antibacterial activity of *Rhizophora mucronata* extracts and content of secondary metabolites ever done. In its phytochemicals have several kinds of compounds such as tannins, alkaloids, flavonoids, terpenoids and sapponins [3] [6] [13]. Based on research conducted said that *R. mucronata* bark extract has antimicrobial properties against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* whereas stems of *Rhizophora apiculata* extract has antibacterial and antifungal properties against...
Candida albicans. Another study conducted said that mangrove leaves extracts have antibacterial properties against Escherichia coli and antifungal against Penicillium digitatum [3] [12].

Based on the foregoing, we intends to continue the research to determine the phytochemical content and antibacterial activity of mangroves R. leaves extract.

II. RESEARCH METHODS

This research is an experimental study that includes sample preparation, extraction (maceration), evaporation, phytochemical test and antibacterial activity test which conducted at the Chemistry and Biology Laboratory, State University of Makassar. Research was conducted in September 2014-January 2015.

The tools used in this research are laboratory glassware, analytical balance, oven, blender, maceration vessel, rotary evaporator, needle ose, Buchner funnel, petri dish, a hot plate, a magnetic stirrer and water bath.

The materials used in this study are R. mucronata leaves, methanol, Whatmann filter paper, Staphylococcus aureus, Escherichia coli bacteria, 96% ethanol, paper disc, a petri dish, Nutrient Agar (NA), aluminum foil, paper filter, tissue, distilled water, napkins and alcohol 70%.

A. Sample Preparation

R. mucronata leaves samples taken from Lappa Village, Samataring District, East Sinjai Regency, South Sulawesi. R. mucronata leaves sampled are still fresh leaves which cleaned and washed and then dried with aerated.

B. Sample Extraction

The process of extraction were done using maceration techniques. A total of 500 grams of dry leaves of R. mucronata macerated with methanol during 2x24 hours. The extract was concentrated using a rotary evaporator to obtain. This extract was used for testing in phytochemicals and antibacterial effectiveness.

C. Test Phytochemicals

Methanol extracts of R. mucronata leaves analyzed to identify the type of secondary metabolites contained in the sample. The reagents used include FeCl$_3$ (phenolic test), Liebermann-Burchard (steroids and terpenoids test), Mayer (alkaloids test), and Wagner (alkaloids test).

D. Antibacterial Activity Test

The initial step in testing the bacteria sterilization of tools and media using the autoclave was set up at a temperature of 121°C at a pressure of 15 psi (per square inch). R. mucronata extracts for antibacterial testing was done by measuring the Inhibitory Power Diameter (IPD) on the growth of Staphylococcus aureus and Escherichia coli.

The method was done by measuring the diameter of the clear zone around the paper disk antibacterial activity. Inhibitory zone diameters obtained were then compared to the negative control inhibition zone (distilled water).

IPD methods performed using filter paper circle that has been soaked in the sample for 1 hour, placed on the medium in a petri dish that has been inoculated with microorganism test. The measured parameter was the area resistor were clear zone formed around the paper disc after incubated for 2x24 hours at a temperature of 37°C. Inhibitory zone diameters measured in millimeters (mm) using calipers by way of reduced overall diameter paper disc diameter of 6 mm. In this study, R. mucronata leaf extract with used 10%, 20%, 40%, 60% concentrations and sterile distilled water was used as a negative dick.

III. RESULTS AND DISCUSSION

This research used methanol to extract the metabolites in a sample. Where, methanol is able to penetrate the cell wall on the sample so that compounds that are polar and non-polar can be extracted in methanol.

Results of research have shown that the methanol extract of R. mucronata leaves positive for some type of secondary metabolites compounds. The reagen tested can be seen in Table 1.
Table 1 Result of Colour Tested To R. mucronata leaves Methanol Extract

<table>
<thead>
<tr>
<th>Regen- Tested</th>
<th>Colour</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₃</td>
<td>Green → brownish green</td>
<td>(+) Flavonoids</td>
</tr>
<tr>
<td>Liebermann-Burchard</td>
<td>Green → clear green</td>
<td>(+) Steroids</td>
</tr>
<tr>
<td>Mayer</td>
<td>Green → green</td>
<td>(-) Alkaloids</td>
</tr>
<tr>
<td>Wagner</td>
<td>Green → brown</td>
<td>(-) Alkaloids</td>
</tr>
</tbody>
</table>

Test results obtained showed that the extract were flavonoid compounds group. It is shown from the positive reaction between two reactants isolates with iron (III) chloride (FeCl₃) 1% which indicated by a color change from green to brownish green.

Identification also showed a steroid compounds in extracts of R. mucronata with technical analysis phytochemical that is characterized by the formation of green color after being given test with Liebermann-Buchard reagents. Identification of the alkaloid in the plant extract made with two reagents phytochemical test, i.e. Mayer and Wagner reagents. At Mayer reagent characterized by the formation of a white precipitate, while Wagner reagent characterized by the formation of brown to yellow. In phytochemical test of R. mucronata leaves extracts does not contain alkaloid compounds, results showed no white formation deposits on the Mayer reagent deposition and brown to yellow on Wagner reagents.

Based on testing that has been done can be seen that the R. mucronata methanol extract positive for some type of secondary metabolites, i.e. steroids and flavonoids. R. mucronata extracts for antibacterial testing was done by measuring Inhibitory Power Diameter (IPD) on the growth of Staphylococcus aureus and Escherichia coli. The method was done by measuring the diameter of the clear zone around the paper disk antibacterial activity. Clear zone is an indication of the bacteria sensitivity to antibiotics or other antibacterial material that was used as the test material which is expressed with a width of inhibition zone diameter. Inhibitory zone diameters measured in millimeters (mm) using calipers by way of reduced overall diameter paper disc of 8 mm diameter. Then the diameter of inhibition zone is categorized as antibacterial power classification [2].

In this study, the concentration of R. mucronata methanol extract used are 10%, 20%, 40%, 60% concentrations and sterile distilled water was used as a negative dick. Antibacterial effectiveness test results are presented in Figure 1 below.

Figure 1. IPD R. mucronata extract against S. aureus and E. coli

10 % and 20 % concentration with positive control

S. aureus 40% and 60% concentration   E. Coli 40% and 60% concentration
Based on the test results of *S. aureus* and *E. coli* against activity, obtained extensive IPD for *S. aureus* at 60% concentrations amounting to 15.12 mm (strong), 40% concentrations amounting to 8.63 mm (medium), 20% concentrations with 1, 33 mm (less), containing 10% concentrations with 0.87 mm. While the bacteria *E. coli*, obtained extensive IPD to 60% concentration with 13.42 mm (strong), 40% concentrations was 6.26 mm (medium), 20% concentrations with 0.91 mm (less), containing 10% concentrations with 0.61 mm and positive control (distilled water) with 0 mm.

Based on these results, it can be seen that the concentration of *R. mucronata* leaves methanol extract which has the greatest inhibition was at 60% concentration with area was 15.12 mm clear zone on *S. aureus* and 13.42 mm in *E. coli* bacteria.

According [2], antibacterial strength criteria as follows: inhibition zone diameter of 5 mm or less categorized as weak, 5-10 mm zone of inhibition is average, 10-20 categorized strong inhibition zone, and the zone of inhibition of 20 mm or more categorized as very strong. The establishment of clear zone around the paper disc shows the inhibition of the growth of bacterial colonies due to the influence of bioactive compounds contained in n-hexane extract of leaves of the soursoap.

At 40% and 60% concentration formed wide clear zone indicating that there has been inhibition of bacterial growth while at 10% and 20% concentration less formed clear zone indicating that the lack of inhibition of bacterial growth on both bacteria represent gram-positive and negative. The inhibition of bacterial growth is due to secondary metabolites class of flavonoids and steroids that are bioactive at a 40% and 60% concentration, whereas the concentration of 10% and 20% also contain secondary metabolites class of flavonoids and steroids but the number of compounds in the extract concentration was slightly, so it can’t to inhibit the growth of bacterial colonies.

The inhibition of the growth of bacterial colonies suspected to be caused due and damage on the structural components of bakteri cell membranes. Other research result suggests that cell membranes are composed of proteins and lipids which particularly vulnerable to chemicals and can reduce the surface tension. Damage to the cell membrane causing disruption of nutrients transport (compounds and ions) through the cell membrane, so that the bacterial cells deprived of nutrients needed for growth.

VI. CONCLUSION

From the research that has been done can be concluded that *R. mucronata* leaves methanol extract positive for some type of secondary metabolite compounds, i.e. steroids and flavonoids compounds. And the antibacterial activity test results with Inhibitory Power Diameter (IPD) measuring showed that a strong antibacterial activity against *S. aureus* and *E. coli* with 60% concentration is due to have a value of IPD > 11 mm and being category for 40% concentration. At the time of the test inhibitory antibacterial activity of *R. mucronata* extract begin to look at 10% to 20% concentration with no sightings in media and there was less clear zone category. The ability occurs because of the role of the chemical compounds in extracts of *R. mucronata* extract of flavonoids and steroids compounds.

V. BIBLIOGRAPHY


