Anti-Methicillin Resistant Staphylococcus aureus (M RSA) of Methanol Extract of Mangrove Plants Leaf: Preliminary Report

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Increasing multidrug resistant (MTDR) in bacteria, in particular Staphylococcus aureus has been becoming worst. Finding new antibiotic source for MTDR is compulsion for scientist and clinicians due to the limitation of the therapeutic option. This research was conducted to investigate the antibacterial activity of methanol extracts of Sonneratia caseolaris, Acanthus ilicifolius, Rhizophora mucronata and Excoecaria agallocha against methicillin resistant, clinical isolates of Staphylococcus aureus using disk diffusion method. The minimum inhibitory concentration (MIC) values of all methanolic mangrove leaves ranged from 6 to 20 mg/mL. The mangrove leaf of Excoecaria agallocha possessed higher anti MRSA potency than the other mangrove plants. It is clear that mangrove leaves especially, E. agallocha leaf has anti MRSA compound. Further purification of active compound from the potent mangrove will be useful for the control of drug resistant strains of S. aureus.

Key words: Mangroves, Antibacterial, Methicillin Resistant Staphylococcus aureus

INTRODUCTION

In recent years, the emergence of multidrug resistant (MTDR) pathogenic bacteria Staphylococcus aureus present crucial problem. Methicillin Resistant Staphylococcus aureus(MRSA) is one of the most important nosocomial pathogen that caused septicemia, suppuration, abscess formation, a variety of infections and even death.[1] Resistan Staphylococcus aureus on methicillin cause increasing the cost of treatment against this infection. Treatment of this infection through antibiotic therapy has become not only problematic but also follows the risk of altering the MRSA pathogen into MTDR strains.

Since there have been no promising new antibiotic agent for MRSA infections, researcher in the microbiology field have been force to gain new drug for it. Traditional medicinal plants are a popular choice for those with those who have compulsion to overcome MRSA infection. Moreover, in the recent decade, many scientists have been continuing to search new anti-MRSA sources to achieve antibiotics from any kind of plant. [2,3,4,5]

Indonesia as one of the high diversity country in the world has enormous medicinal plants. One of the medicinal plants are often used by people of Indonesia on traditional medicine is the mangrove. [6] Mangroves are woody trees or shrubs. The mangrove plants are distributed in 121 countries. Mangrove forest is one of the coastal ecosystems of Porong, Sidoarjo, Indonesia with rich vegetation. Numerous mangroves are used in traditional medicine for the treatment of many diseases. [7,8]

The mangrove leaves have also been proved for anti-ulcer properties,[9] antiplasmodial,[10] antibacterial,[11] antiviral,[12] and antioxidant. [13] In this present study, four mangrove plants leaves were screened for their antimicrobial activity against MRSA.

MATERIALS AND METHODS

Plants material: The leaves of four mangrove species: Sonneratia caseolaris, Acanthus ilicifolius, Rhizophora mucronata and Excoecaria agallocha were collected from the mangrove forest in Porong river bank, Sidoarjo, Indonesia during December, 2011 and used for the present investigation. Plants were authenticated by the botanist from Department Biology, Faculty of Mathematic and Life Science, Brawijaya University, Indonesia. The leaves were rinsed with distilled water and packed in Poly ethylene plastic. The leaves were then eventually brought to laboratory.

Microorganism used

Clinical sample of MRSA strain is received from the Laboratorium of Microbiology, Faculty of Medicine, Brawijaya University, Indonesia.

Extract preparation

The methanol extract were prepared by stirring 50 g of the powdered mangroves leaves in 500 ml methanol (Merck, Darmstadt, Germany) for 24 hour. The extracts were filtered using whatman filter paper no. 1 and were then concentrated using rotary evaporator at 50°C.

Antibacterial assay

The agar diffusion Bauer method[14] was used for antibacterial susceptibility test with slight modification. Blank disks (Oxoid, Hampshire, UK) were impregnated with 20 μl of the extracts (concentration: 100 mg/ ml). 5% aqueous DMSO was used as negative control and standard antibiotic Vancomycin (30 μg/ml) was used as positive control. The disks were then put onto Mueller Hinton Agar (MHA) (Merck, Darmstadt, Germany) that have seeded with 1.5 x 106 CFU/ ml MRSA. Inhibitory zones (including the diameter of disk) on the agar surface around the disks was measured.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined as the lowest concentration of the crude extract in the broth medium that inhibited the visible growth of MRSA. The well plates were filled with 100 μl of 1.5 x 106 CFU/ ml MRSA in Mueller Hinton Broth (Merck, Darmstadt, Germany). Afterward, 20 μl of the extract concentrations of 1.0 to 100
mg/ml were inoculated into each well containing MRSA. The plate was then incubated at 37°C for 24 hour. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

The range of mean zone of inhibition was between 6.6 and 11.4 mm. The inhibition zone of four mangrove plants were almost of the entire produced a mean inhibition zone of more than 7 mm, except for A. illicifolius that was slightly less than 7 mm (Table-1). The zones of inhibition produced by the positive control, vancomycin is 14.2 mm. The negative control, 5% aqueous DMSO did not produce any zone of inhibition. The MIC values were between 6 and 30 mg/ml. The lowest MIC value obtained from methanol extract of E. agallocha leaf. 

Table 1: Anti MRSA of methanolic extract of mangroves plants leaf.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Samples</th>
<th>Zone of inhibition (mm)</th>
<th>MIC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. caseolaris</td>
<td>7.5 ± 0.87</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>A. illicifolius</td>
<td>6.6 ± 0.96</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>R. mucronata</td>
<td>8.8 ± 1.01</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>E. agallocha</td>
<td>11.4 ± 0.77</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Positive control</td>
<td>14.2 ± 0.68</td>
<td>Nt</td>
</tr>
<tr>
<td>6</td>
<td>Negatif control</td>
<td>nd</td>
<td>Nt</td>
</tr>
</tbody>
</table>

include diameter of disk (6 mm); ± = standard deviation; nd = not detected; Nt = not tested.

In this study revealed that the methanol was the effective solvent for extracting anti-MRSA compound. This result agreed with Chandrasekaran research. He found that mangroves like R. mucronata, R. lamkarkii and Bruguiera cylindrica had antibacterial activity against MRSA. The similar result using methanol was also showed by Yogisha and Raveesha.

The bioactives from plants that have biological activities or chemical constituents is important mainly for the discovery of new therapeutic agents. The leaf of mangrove E. agallocha is one of the best resources for bioactive properties to cure MRSA. On the other hand Chandrasekaran research revealed that Rhizophora leaf is the best source to combat MRSA. This difference was probably because of the habitat of the mangroves. The samples of this study were taken from Porong river bank that have been contaminating by Lapindo Brantas Inc. wastewater within six years. The production of bioactive substances by plant is related to several factors, such as independent evolution, genetic, and climate.

Based on the result of antibacterial susceptibility testing against MRSA, mangrove plant leaves could be considered to be rich sources of novel anti-MRSA even though one species (A. illicifolius) showed small zones of inhibition. This result could be due to low concentration of the compounds and the fact that the extract for this study is still in crude form. It was clear that all mangroves (Sonneratia caseolaris, Acanthus illicifolius, Rhizophora mucronata and Excoecaria agallocha) methanolic extracts indicated anti-MRSA. However, the most potential anti MRSA compound was found on E. agallocha leaf. Hence, further research needs to be done to purify and to characterize anti-MRSA compound from E. agallocha.

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REFERENCES


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