PROCEEDING

INTERNATIONAL SEMINAR

2nd DENTISPHERE.

"Current Concept in Dentistry"

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THE POTENCY OF Rhizophora mucronata’s BARK EXTRACT IN INHIBITING THE GROWTH OF Streptococcus mutans COLONY

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ABSTRACT

Background: Streptococcus Mutans cariogenicity is based on the ability to produce and tolerate large amounts of acid. Rhizophora mucronata have broad spectrum antibacterial properties that can inhibit the growth of gram-positive or gram negative bacteria.

Purpose: The objective of this study was to examine Minimum Inhibition Concentration (MIC) against Streptococcus mutans.

Methods: Subjects were 32 samples of S.mutans, divided into 8 groups (n = 4). Six groups were given the extract with different concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, control positive group was given eugenol 0.025mg/ml and control negative was given 1% DMSO. Extracts was prepared by percolation method, sample of bacteria were inoculated from patients’s saliva. The inhibitory effect was observed by measuring the diameter of inhibition zones (clear zone) on agar media with digital calipers. Data were analyzed with kolmogorov smirnov and one-way anova

Result: There were significant difference between each concentration (50%-p=0.015; 25%-1.56%-p=0.000) and control positive. There were significant difference between concentration (50%-p=0.000; 25%-p=0.001; 1.56%-p=0.001) and no significant difference between concentration (12.5%-p=0.083; 6.25%-p=0.091; 3.125%-p=0.054) and control negative.

Conclusion: Rhizophora mucronata’s extract has inhibition potency against Streptococcus mutans. The minimum inhibitory concentration (MIC) is 50% in bark’s stem extract.

Keywords Rhizophora mucronata, Streptococcus mutans, minimum inhibitory concentration

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INTRODUCTION
Dental caries is one of the common diseases that infect humans. Caries is a process of remineralization and demineralization cycle with various stages of being either reversible or irreversible. Tooth decay is the result of subsequent demineralization of enamel and dentin with acidic products produced by plaque microorganisms from the metabolism of carbohydrates. However, the initial process of demineralization is usually accompanied by remineralization. When the surface layer of the enamel has gone, the infection will continue to the dentin, and the pulp will inflamed and become necrotic. Dental caries is an active lesions that sometimes also called smooth surface caries lesions or white spot. Dental caries is the major cause of tooth loss before age 35 years. Streptococcus mutans (S. mutans) is an opportunistic commensal oral bacteria that play an important role as the main cause of caries. These bacteria have the ability to stick to the tooth surface, colonizes, and causing caries.

Indonesian Mangrove Center in 2006 showed that Indonesia is one of country that has the largest mangrove forest in the world. Extensive mangrove forests in Indonesia reaches 25% of the total 18 million hectares of mangroves in the world. Mangroves as coastal flora has some content that can be used in alternative medicine. One species is Rhizophora mucronata. Rhizophora mucronata easily found because it spreads from the outer coast waters to areas that were flooded sand. Based on research by Gaharu, 2013, Rhizophora mucronata’s extract have antibacterial potency against dental bacteria not only against Streptococcus mutans. Inside the Rhizophora mucronata’s leaves and bark’s stem there is a high content of active chemicals including tannins. Tannins, in low concentrations can inhibit the growth of bacteria, whereas at high concentrations, tannins worked as antimicrobial and coagulating protoplasmic germs, thus forming a stable bond with germ protein. Beside tannins, there are some other ingredients in the bark and leaves of the Rhizophora mucronata that has an antibacterial potency such as alkaloids, saponins, flavonoids, and terpenoids.

The aim of this study is to knowing the antimicrobial potency, MIC (Minimum Inhibitory concentration) of Rhizophora mucronata’s bark stem extract in several concentrations: 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56% in inhibiting the growth of Streptococcus mutans.

MATERIAL AND METHODS
Extraction of Rhizophora mucronata. The bark’s stem taken from the outermost to the part before the cambium. 10 grams for each variable of fresh Rhizophora mucronata’s bark stem were taken then washed them with tap water and rinsed with sterile distilled water. We use
fresh or wet extraction method. The wet extracts's process was without the drying process, it
was only dried up the distilled water on the surface of the bark stem. After that cut them into
small pieces and mashed in a blender, then soaked in 83% ethanol in 500 ml Erlenmeyer for 3
and 6 hours Put the filtrate in the falcon tube then centrifuged for 15 minutes at 7000 rpm.
The results form are supernatant and pellet, collected the supernatant in Erlenmeyer and
discarded the pellet. Evaporation process was performed using an evaporator to evaporate the
ethanol that contained in the extract. Leaf and bark’s stem extract diluted with distilled water
to get the variation of the concentration: 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%.
Eugenol 0.025mg/ml was given as the positive control and DMSO 1% was given as the
negative control.

Isolation of Streptococcus mutans. Samples are taken from patient’s saliva in Dental
Hospital of Hang Tuah Surabaya University. The breeding of Streptococcus mutans using
TYC medium. Before breeding, the saliva diluted 3 times using sterile BHI, each tube
contains 4.5ml of sterile BHI. Took 0.5 ml from The solution which was diluted to 10³ then
pour it on TYC medium then leveled with a spreader. Put the media in an anaerobic jar and
then stored it in an incubator at a temperature of 37⁰C for 1x24 hours.

Inhibition test of Rhizophora mucronata’s extract against bacterial growth of
Streptococcus mutans. Dip the filter paper Ø 6mm which previously soaked in Rhizophora
mucronata’s extract for 10 seconds in the treatment group. For the negative control group,
filter paper soaked in dmsos 1% for 10 seconds, while for the positive control, using eugenol.
The filter paper laid on Streptococcus mutans media using sterile tweezers the pressed. Put
the petri dish to the incubator for 2x24 hours at 37⁰C in anaerobic atmosphere. Measuring the
inhibitory zone of Rhizophora mucronata’s extract is by measuring the clear area around the
filter paper using digital calipers (in mm). The measurement is performed on the last clear
boundary on the most left side colony until the last clear boundary on the most right side
colony which is measured the distance of the longest clear area. Clear zone result were
classified in the following table (table 1).

The MIC (Minimum Inhibitory Concentration) test of Rhizophora mucronata’s Extract
Against the growth of Streptococcus mutans. The group concentrations of Rhizophora
mucronata’s extracts that we use were 50%, 25%, 12.5%, 6.25%, 3,125%, and 1,56%. One
group of positive control (K +), and one group of negative control (K-). Each group performed repetitions over three times. All of the tubes were incubated at 37 °C for 24 hours, then observed and compared with the positive control. The smallest sample concentrations can inhibit bacterial growth (observed visually by three observers) was determined as the Minimum Inhibitory concentration (MIC).

<table>
<thead>
<tr>
<th>Diameter of The Clear Zone</th>
<th>Growth Inhibition Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10mm</td>
<td>None</td>
</tr>
<tr>
<td>11 mm – 15mm</td>
<td>Weak</td>
</tr>
<tr>
<td>16 mm – 20 mm</td>
<td>Average</td>
</tr>
<tr>
<td>&gt; 21 mm</td>
<td>Strong</td>
</tr>
</tbody>
</table>

RESULTS

The clear zone formed on the antibacterial activity of the diffusion method showed there’s potency of Rhizopora mucronata’s bark stem extracts in all concentration against Strptococcus mutans. The longest clear zone found in Bark’s stem extract at concentration 50%. (Fig 1)

![Fig 1. Graphic of The Clear Zone Diameter of Rhizopora mucronata’s Extract Against the Growth of Streptococcus mutans](image)

Based on the classification of growth inhibition response by greenwood, the growth inhibition response of Rhizopora mucronata’s extract against Streptococcus mutans is low. Even the extract have low antibacterial potency, there are some significance different between each groups. The analysis results (one way Annova) with the level of 95% showed in table.
The table showed there were significant difference between each concentration and control positive. There were also significant difference between concentration at 50%, 25%, and 1.56%, but no significant difference between control negative and concentration at 12.5%, 6.25%, 3.125%.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Groups (i)</th>
<th>Groups (j)</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>BARK STEM</td>
<td>K+</td>
<td>50%</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td>25%</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5%</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.25%</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.125%</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.56%</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td>K-</td>
<td>50%</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25%</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.5%</td>
<td>0.083</td>
<td></td>
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<tr>
<td></td>
<td>6.25%</td>
<td>0.091</td>
<td></td>
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<tr>
<td></td>
<td>3.125%</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.56%</td>
<td>0.001*</td>
<td></td>
</tr>
</tbody>
</table>

*significance

DISCUSSION

In our research, 50% bark’s extract is a weak inhibitor against Streptococcus mutans due to The Classification of Growth Inhibition Response by Greenwood, 1995 (table1). Compared with the positive control, which is eugenol, they have significant difference in all concentration. Eugenol has strong antibacterial potency. Some previous in vitro research showed that eugenol has antibacterial effect. Antibacterial mechanism such as interfere membrane cell’s permeability, inhibiting the synthesis of cell wall, inhibiting the function of membrane cytoplasm and inhibit the growth of bacteria especially Streptococcus mutans that cause dental caries at a concentration of 0.025 mg/mL.

We use dmso 1% as the negative control because it is a good solvent. The use of low concentration because dmso also has antibacterial potential. There is significant difference between negative control and 50%, 25%, and 1.56% concentration of bark’s extract. There is no significant difference between negative control and 12.5%, 6.25%, and 3.125% concentration of bark’s extract. Maybe the significant difference that found at 1.56% concentration caused by the bond between the concentration and the dmso, but to make sure what caused that significant difference it needs further research.
Although they have a weak antibacterial potency it still has advantage of being derived from natural ingredients.

CONCLUSION

Rhizopora mucronata’s extract has inhibition potency against Streptococcus mutans. The minimum inhibitory concentration (MIC) is 50% bark’s stem extract. Need more research to found other potency in Rhizopora mucronata / mangrove plants.

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