In Vitro Antimicrobial Activity of Propolis on Mutans of *Streptococcus mutans* Isolated from Human Harbouring Species in Bangka Island

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ABSTRACT
Propolis is a natural beehive product, and its main constituents are polyphenolic compound, such as flavonoids and cinnamic acid derivatives. **Methods:** Propolis from Parung Panjang West of Java, Indonesia was examined for in vitro to inhibit the bacterial growth by determining the inhibitory zone, minimum inhibitory (MIC), and minimum bactericidal concentration (MBC). The microorganisms tested were: Mutans of *Streptococcus mutans* isolated from human harbouring species in Bangka Island Indonesia labeled as: S.mutans1, S.mutans2, S.mutans3, S.mutans4, S.mutans5 and S.mutans6 The concentration of Propolis polyphenolic compound used 62.5 µg/ml Propolis polyphenolic. The statistical analysis was done by in a descriptive. **Results:** The MIC values were 31.25 µg/ml, average of inhibition zone 3.00 mm for S.mutans1; 15.62 µg/ml, average of inhibition zone 1.50 mm for S.mutans2; 15.62 µg/ml, average of inhibition zone 2.00 mm for S.mutans3; 31.25 µg/ml, average of inhibition zone 1.90 mm for S.mutans4; 15.62 µg/ml, average of inhibition zone 1.75 mm for S.mutans5; 31.25 µg/ml, average of inhibition zone 1.50 mm for S.mutans6 and their MBC value was higher than MIC. **Conclusion:** This study shows that Propolis polyphenolic compound has antimicrobial activity against mutans streptococci.

Key words: Propolis, Mutans streptococci, Species

Introduction
Dental caries is chief dental health problem throughout the world although its prevalence in several industrial countries has declined. Opposite condition occurs in Indonesia, caries prevalence tends to increase. In fact the people suffering from tooth ache is are on the six rank of common disease found in Indonesia.

Regardless of the prevention against the increasing of dental caries has been conducted by various methods, such as tooth brushing by toothpaste, mouth washing, antibiotic etc, including suggestion through electronic media, newspaper, magazine etc, dental caries still showed a high figure.

Recently there is wide spread that honey beehive contains active elements: propolis which can cure various disease. However whether propolis use is medically accountable, needs careful and profound study to know exactly the actual effect of propolis. Propolis has been found to inhibit the synthesis of protein by bacteria, which may account for at least some of its antimicrobial effects. In this study is to evaluate propolis which is used to maintain health and to examine the effect of antimicrobial activity of Propolis on mutans streptococci.
The objective of this research is determining the inhibitory zone, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on mutans streptococci.

The research result will provide information about propolis and its effectiveness in preventing dental caries for dentistry in particular and for researcher in general. This will hopefully lead another research that eventually will produce a new, save and inexpensive in dental treatment.

Material and Methods

The material used in this research is propolis which is derived from Parung Panjang Bee’s Plantation Bogor, West of Java Indonesia, in the form of Ethanolic Extract of Propolis (EEP) with concentration of 62.5 µg/ml. EEP was done in the Chemistry Laboratory of Faculty Mathematics and Science University of Indonesia.

The bacteria used as analysis unit were local strains of mutans of Streptococcus mutans isolated from human harbouring species in Bangka Island Indonesia labeled as S.mutans1, S.mutans2, S.mutans3, S.mutans4, S.mutans5 and S.mutans6.

Mutans of Streptococcus mutans are cultivated in Tryptose-Yeast Sucrose with Bacitracin (TYS20B). Brain Heart Infusion Broth (BHI), and Diagnostic Sensitivity Test (DST) was performed. Those specimen are incubated in anaerobic jar at 370 Celsius degree for 3 X 24 hours.

Sensitivity test to antibiotic can be done in two ways:

A. Using disc with drug in solid media method.
B. Drug serial dilution method.

A.1. Using Disk with Drug in Solid Media Method

A.1.1. Making of the Bacteria Culture Medium

1. From the cultivated Streptococcus mutans bacteria, take one loop of bacteria, and then re-cultivated them in TYS20B culture medium. The tube is put inside the incubator in an anaerobic jar at 37 Celsius degree for 72 hours.
2. If the Streptococcus mutans which has grown from the TYS20B culture medium will be taken one loop and then put it in BHI liquid culture medium. The tubes will be put inside the anaerobic jar at 370 Celsius degree for 72 hours.
3. After the incubation, compare the turbidity of bacteria medium culture in BHI with McFarland II standard solution.
A.1.1.2. Bacteria Dilution

If the turbidity of *Streptococcus mutans* culture medium is equal to McFarland II solution, then culture of *Streptococcus mutans* shall be diluted as follows:

1. Take 1 ml from the tube that contains bacteria, then put it in a tube contains 9 ml sterile physiologic salt solution (tube A). Then mixed them until homogenized of 10 times of dilution.
2. From tube (tube A) take 1 ml of solution and put it in a tube that contains 9 ml of sterile physiologic salt solution (tube B). Then mix them until homogenized of 100 times of dilution.
3. From tube B, take 1 ml of solution and put it in a tube contains 4 ml sterile physiologic salt solution (tube C). Then mix them until homogenized. of 500 times of dilution.

A.1.1.3. Bacteria Cultivation on DST agar

1. Put each I ml of diluted *Streptococcus mutans* in the agar DST petri disk. Put them in a motion so that the bacteria suspension wet the DST agar thoroughly.
2. Then put 5 ug/ml propolis on a disk, and put it on the surface of DST agar.
3. Incubate the petri disk inside an anaerobic jar at $37^0$ Celsius degree for 72 hours.
4. After 72 hours, check the medium with tested samples by measuring the diameter of the isolated zone around the samples (Inhibition zone).

B. Drug Serial Dilution Method

B.1. Making of Bacterial Culture

1. Take one loop of *Streptococcus mutans* respectively from preserved solid agar culture TY20SB and slanting blood agar and grow them in BHI liquid media tubes.
2. The tubes are incubated at $37^0$ Celsius degree for 72 hours in anaerobic jar.
3. After 2-3 days, compare turbidity of bacterial culture in BHI tubes with McFarland II standard solution. If bacterial culture in BHI tubes is more turbid, add sterile physiologic saline solution little by little until their turbidity is equal to turbidity of McFarland II standard solution.
4. If turbidity of bacterial culture is equal to turbidity of McFarland II standard solution, total number of *Streptococcus mutans* in it is more or less $609 \times 10^6$ bacteria/ml.
B.1.2. Bacteria dilution

1. Put 5 ml of calibrated bacterial culture to test tube and then put 5 ml of physiologic saline solution.
2. Then dilute calibrated *Streptococcus mutans* culture until $10^6$. The dilution shall be done 7 times so the last tube is estimated to contain 50 bacteria cells per ml.

B.1.3. Sensitivity Test to EEP

1. Prepare 8 test tubes containing 2 and 4 ml propolis solution plus BHI, add 1 ml of *Streptococcus mutans* culture result of the last dilution to respective tubes.
2. The eight tubes are incubated at $37^\circ$ Celsius degree for 72 hours, also incubated is in standard solution without addition of bacterial culture and without propolis.
3. After 3 days, we examine the tubes macroscopically, and find out at what sequence number of tube the bacteria can not grow.
4. Record the result the minimum inhibitory concentration (MIC) Then test the minimum inhibitory concentration (MIC) in blood agar plate.

Data obtained is analyzed in a descriptive.

Results

The results are as follows:
The result of inhibitory zone measurement on DST agar plate and sensitivity test to EEP can be seen in tables as seen below:

Table 1 The result on inhibitory zone measurement in bacterial growth of *mutans* of *Streptococcus mutans* on DST agar media.

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Inhibitory zone in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus mutans1</em></td>
<td>3.00</td>
</tr>
<tr>
<td><em>Streptococcus mutans2</em></td>
<td>1.50</td>
</tr>
<tr>
<td><em>Streptococcus mutans3</em></td>
<td>2.00</td>
</tr>
<tr>
<td><em>Streptococcus mutans4</em></td>
<td>1.90</td>
</tr>
<tr>
<td><em>Streptococcus mutans5</em></td>
<td>1.75</td>
</tr>
<tr>
<td><em>Streptococcus mutans6</em></td>
<td>1.50</td>
</tr>
<tr>
<td>Mean</td>
<td>1.95</td>
</tr>
</tbody>
</table>

From the measurement of inhibitory zone which is carried out from the border of disk to zone with bacterial growth showed that the average in inhibitory zone of 62.5 $\mu$g/ml is 1.95 mm.
Table 2. The result of serial dilution test of mutans of *Streptococcus mutans* to Ethanol Extract of Propolis (EEP)

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>The concentration of EEP (µg/ml)</th>
<th>C(+)</th>
<th>C(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>62.5</td>
<td>31.25</td>
<td>15.62</td>
</tr>
<tr>
<td><em>S. mutans</em>1</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td><em>S. mutans</em>2</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em>3</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em>4</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em>5</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em>6</td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

+ growing  - not growing  C(+) Positive control  C(-) Negative control

The result of sensitivity test of *Streptococcus mutans* to Propolis dilution series shows that all specimens in tubes IV, V, VI, VII, VIII are fully turbid, three of bacteria grew on the third tube (tube III) there is no turbidity at tube number I and II. From the above table we find out that minimum inhibitory concentration (MIC) of EEP on *Streptococcus mutans* are 31.25 µg/ml /15.62 µg/ml and MBC is 62.5 µg/ml.

**Discussion**

From the foregoing research that Propolis can inhibit the growth of *Streptococcus mutans*1; *Streptococcus mutans*2; *Streptococcus mutans*3; *Streptococcus mutans*4; *Streptococcus mutans*5 and *Streptococcus mutans*6 in vitro, inhibitory zones are 3.00, 1.50, 2.00, 1.90, 1.75, 1.50 mm and the Minimum Inhibitory Concentration are 31.25 µg/ml/15.62 µg/ml and MBC is 62.5 µg/ml.

In the past study it had been proven that Ethanolic Extract of Propolis (EEP) with concentration of 62.5 µg/ml has anti microbial activity against local strains of mutans of *Streptococcus mutans* isolated from human harbouring species and standard strains of *Streptococcus mutans* KPK2, *Streptococcus mutans* Ing Britt and *Streptococcus sobrinus* B13.

Propolis is produced by bees all over the world, its chemical composition depends on the plant ecology of the specific regions where the bees live.

Propolis has over 38 compounds called flavonoids, flavonoids are actually plants natural pigments and active components of propolis, which account for most of its usefulness. Propolis also contains a various types of phenolic such as cinnamyl alcohol, cinnamic acid, vanillin, benzyl alcohol, benzoic acid and caffeine ferulic acid.

From those properties of Propolis mentioned above that Propolis has a potential anti microbial activity as stated by many scientific tests have been conducted with a variety of bacteria, fungi, and viruses. It also stated that propolis can reduce dental caries by reducing plaque formation.
This study supports previous research that Ethanolic Extract of Propolis (EEP) type 3 and type 6 originating from Brazil Atlantic can inhibit the growth of *Streptococcus mutans* Ing-Britt and *Streptococcus sobrinus* 6715.

Further characterization of the structure and function of the active component of propolis may lead to a new anti-caries product. So it can be used to anticipate caries risk in the future. Propolis has antimicrobial activity on mutans streptococci in vitro, it can be used to anticipate the risk of caries.

Recently, there is widespread that honey beehive contains active elements: propolis which can inhibit the growth of mutans streptococci. However, whether propolis use is medically accountable needs careful and profound study to know exactly the actual effect of Propolis.

**Acknowledgement**

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**References**

5. Mangundjaja S and Soekanto S.A. In Vitro Antimicrobial Activity of Propolis on Mutans of *Streptococcus mutans* Isolated from Schoolchildren Harbouring Species in Riau Island Indonesia. Paper read at The IADR of Asia Pacific Section Meeting 3-6 September 2004. Samui Island Thailand
   Available at http://www.nidr.nih.gov/news/digest/may01-1.asp, 2001
   Available at http://www.vespapower.com/propolis.htm, 2001

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