In vitro Antimicrobial Activity of Green Tea Polyphenol on Mutans Streptococci

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Abstract
The aim of the study was to determine the sensitivity of polyphenol green tea. Tea is a traditional drink, which is beneficial for health, one of its benefits is capability to prevent dental caries because it has active substance polyphenol. An extract of Green tea non-fermented tea leaves of *Camellia sinensis* and its chromatographically isolated polyphenol compound was examined in vitro to inhibit the bacterial growth by determining the inhibition zone (agar diffusion method), minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). The microorganisms tested were: *Streptococcus mutans* Ing-Britt, *Streptococcus mutans* KPSK2, *Streptococcus mutans* JC2, *Streptococcus mutans* LM7 and *Streptococcus sobrinus* B13. Results showed Green tea polyphenol was the most effective against *Streptococcus mutans* Ing-Britt (Inhibition zone 3.60 mm; MIC 10^{-2}/ml, MBC 10^{-1}/ml); *Streptococcus mutans* KPSK2 (Inhibition zone 3.10 mm; MIC 10^{-3}/ml, MBC 10^{-1}/ml); *Streptococcus mutans* JC2 (Inhibition zone 2.98 mm; MIC 10^{-2}/ml, MBC 10^{-1}/ml); *Streptococcus mutans* LM7 (Inhibition zone 3.00 mm; MIC 10^{-3}/ml, MBC 10^{-1}/ml) and *Streptococcus sobrinus* B13 (Inhibition zone 2.05 mm; MIC 10^{-1}/ml, MBC 10^{-1}/ml) tea polyphenol. Thus, we concluded that Green tea leaves polyphenol has antimicrobial activity against *mutans streptococci*.

Key words: Green tea polyphenol, *mutans streptococci*

INTRODUCTION

Dental caries is a main dental health problem throughout the world although its prevalence in several industrial countries has declined, on the other hand in Indonesia, caries prevalence tends to increase. This condition is a challenge for dentistry profession to prevent the dental caries. *Streptococcus mutans* is the main cause of dental caries by triggering dental plaque formation. An effort to control dental caries in the community has been done in many ways, such as tooth brushing, mouth rinsing with antiseptic, adding fluoride or several enzymes into toothpaste, and fluoridation at drinking water in certain community, but actually dental caries is high.

Tea is commonly known available in the market. In dentistry, the most important effect of tea can reduce caries incidence of tea drinkers and it can also adhere mineral onto enamel again, because it contains fluoride.
In recent study “Awur “ tea can reduce the growth of salivary mutans streptococci and also reduce caries activity of mutans streptococci in plaque. It is known that tea has an active component which is called polyphenol compound.

Therefore is still necessary to perform further research to determine antibacterial property of polyphenol in tea leaves in controlling of dental caries.

The objective of this study is to determine the sensitivity of polyphenol Green tea leaves by measuring the inhibition zone, minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) on mutans streptococci, which is done as in vitro.

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

MATERIALS AND METHODS

The material used in this study is an extracted substance from Green tea leaves of Camellia sinensis with fluoride of 3.266 ppm. The bacteria used as analysis unit were Streptococcus mutans Ing-Britt, Streptococcus mutans KPSK2, Streptococcus mutans JC2, Streptococcus mutans LM7 and Streptococcus sobrinus B13.

Mutans streptococci 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 37°C for 3 X 24 hours.

Sensitivity test to antibiotic was done in two ways:

a. Drug serial dilution method:
   1. Making the bacteria culture medium
   From the cultivated mutans streptococci in TYS20B take one loop of bacteria and cultivated them in liquid medium BHI, then incubated it in anaerobic jar at 37 Celsius degree for 2 X 24 hours.
   After 2 days, compare the turbidity of bacteria media culture of BHI with Brown III standard solution.
   If it was found that bacteria culture in BHI media was more turbid, add sterile saline solution, until the turbidity was equal to Brown III standard solution.
   If the turbidity of bacteria culture medium was equal to Brown III standard solution, the number of bacteria cell/ml in bacteria culture medium can be counted, their number is $9 \times 10^8$ bacteria/ml.
2. Bacteria dilution

If equalization process has been done, culture of mutans streptococci bacteria shall be diluted as follows:
Prepare 7 tubes of each 9 ml containing physiologic saline solution and also prepare 1 tube containing 5 ml physiologic saline solution.
Take 1 ml bacteria culture medium then put into the first tube, shake them thoroughly, from the first tube take 1 ml bacteria culture and put into the second tube and do the same thing through the seventh tube.
Take 5 ml of bacteria from the seventh tube and put them into the eight tube and shake it thoroughly.
The number of bacteria is estimated + 50 cells/ml, each tube shall be labeled.

3. Sensitivity test of bacteria to polyphenol

Prepare 5 sets of test tubes, each tube is filled with 9 ml BHI media and is labeled 1 - 5 respectively.
Put 1 ml polyphenol extract as much with 1 : 1 concentration into the first tube then stir well.
From the first tube, take 1 ml of solution and put into the second tube, do the same thing through the fifth tube.
After dilution finished, the put 1 ml of 50 cells of bacteria in those five test tube. All test tubes put in anaerobic jar at 37°C for 2 X 24 hours.
After 72 hours, macroscopically we can see in which tube the bacteria can not grow.
Record the result to determine the Minimum Inhibition Concentration (MIC)

b. Method using disk with drug in solid media

Diluted 1 ml of mutans streptococci in the agar DST petri disc, the bacteria suspension wet the DST agar thoroughly.
Then put polyphenol on a discs and put it on the surface of DST agar.
Those petri discs are incubated in anaerobic jar at 37°C for 3 X 24 hours.
Inhibition zone will show around the disc and measuring the diameter of the isolated zone around the samples.

Data obtained was analysis in a descriptive method.

Results

The results of sensitivity test of Streptococcus mutans Ing-Britt, Streptococcus mutans KPSK2, Streptococcus mutans JC2, Streptococcus mutans LM7 and Streptococcus sobrinus B13 to Green tea polyphenol with serial dilution method can be seen on table 1 and the inhibition zone can be seen on table 2.
Table 1 it showed The Minimum Inhibition Concentration (MIC) is 10^{-2}/ml, MBC 10^{-1}/ml for *Streptococcus mutans* Ing-Britt; MIC is 10^{-3}/ml, MBC 10^{-1}/ml for *Streptococcus mutans* KPSK2; MIC is 10^{-2}/ml, MBC 10^{-1}/ml for *Streptococcus mutans* JC2; MIC is 10^{-3}/ml MBC 10^{-1}/ml for *Streptococcus mutans* LM7; MIC and MBC is 10^{-1}/ml for *Streptococcus sobrinus* B13.

Table 1. Results of sensitivity test of *mutans streptococci* to polyphenol extract in Green tea leaves

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>The concentration of Polyphenol(/ml)</th>
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<tbody>
<tr>
<td></td>
<td>0.1 (I)</td>
</tr>
<tr>
<td><em>S. mutans</em> Ing-Britt</td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em> KPSK2</td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em> JC2</td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans</em> LM7</td>
<td>-</td>
</tr>
<tr>
<td><em>S. sobrinus</em> B13</td>
<td>-</td>
</tr>
</tbody>
</table>

+ growing - not growing  C(+) Positif control (without polyphenol)  C(-) Negative control (with polyphenol)

Table 2 The measurement of inhibition zone which is carried out from the border of disc to zone with bacterial growth showed that inhibition zone of *Streptococcus mutans* Ing-Britt is 3.60 mm; 3.10 mm for *Streptococcus mutans* KPSK2; 2.98 mm for *Streptococcus mutans* JC2; 3.00 mm for *Streptococcus mutans* LM7 and 2.05 mm for *Streptococcus sobrinus* B13.

Table 2. The result on inhibitory zone measurement in bacterial growth of *mutans streptococci* 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 mutans</em> Ing-Britt</td>
<td>3.60</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em> KPSK2</td>
<td>3.10</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em> JC2</td>
<td>2.98</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em> LM7</td>
<td>3.00</td>
</tr>
<tr>
<td><em>Streptococcus sobrinus</em> B13</td>
<td>2.05</td>
</tr>
</tbody>
</table>

DISCUSSION

mutans LM7 are sensitive to concentration $10^{-2}$/ml, while Streptococcus sobrinus B13 is not sensitive to concentration $10^{-2}$/ml Green tea polyphenol.

In the past study it had been prove that an extract Polyphenol of Green tea leaves of Camellia sinensis could inhibit the growth of local strains of mutans streptococci.

Tea leaf contains several chemical substance classified as : phenol substance, non-phenol substance, aromatic compound and enzyme. Tea leaf has been considered a having property of anti microbial against mutans streptococci as stated that one of the most important effect of tea is its capability to decrease the incidence of dental caries of drinkers, and it can also adhere minerals into enamel again, because it contains fluoride.

The crude tea polyphenol compound from the leaf Camellia sinensis were found effectively inhibit the attachment of mutans streptococci to saliva coated hydroxyapatite and could inhibit the growth of mutans streptococci, in vitro.

Tea is a traditional drink from ancient time, but some people also use tea as traditional medicine to cure certain diseases, so generally tea used as daily drink and for preventing some diseases.

CONCLUSION AND SUGGESTION

The result showed that an extract polyphenol from Green tea leaves Camellia sinensis has bacterial activity against Streptococcus mutans Ing-Britt, Streptococcus mutans KPSK2, Streptococcus mutans JC2, Streptococcus mutans LM7 and Streptococcus sobrinus B13.

Because tea contains polyphenol antiseptic, so it can also be used as mouth wash to reduce the population of bacteria in oral cavity so early infection can be anticipated. It suggested to gargle the tea drink before swallowed.

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