Abstract

Objectives: 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. Methods: An extract of Black tea fermented tea leaves of *Camellia sinensis* and its chromatographically isolated polyphenol compound was examined for in vitro to inhibit the bacterial growth by determining the inhibitory zone (agar diffusion method), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The microorganisms tested were: *Streptococcus mutans* Ing-Britt, *Streptococcus mutans* KPSK2, *Streptococcus mutans* LM7 and *Streptococcus sobrinus* B13. The statistical analysis was done by in a descriptive. Results: The Black tea polyphenol was the most effective against: *Streptococcus mutans* Ing-Britt (Inhibition zone 4.50 mm; MIC 6.25%/ml ;MBC 50%/ml); *Streptococcus mutans* KPSK2 (Inhibition zone 1.40 mm ;MIC 6.25%/ml ;MBC 50%/ml); *Streptococcus mutans* LM7 (Inhibition zone 4.30 mm ;MIC 62.25%/ml ;MBC 50%/ml) and *Streptococcus sobrinus* B13(Inhibition zone; 4.50 mm ;MIC 6.25%/ml ;MBC 50%/ml) tea polyphenol. Conclusion: This study shows that Black tea polyphenol has antimicrobial activity against mutans streptococci, *in vitro*.  

Key words: Gopek Tea, Infusum, Mutans Streptococci

Introduction

The tendency of increasing dental caries, in carbohydrate intake from 7.4 kg annually in 1974 to 12.5 kg annually in 1979 to 30 kg annually in 2003 in Indonesia. The Directorate of Oral Health of the Indonesian Ministry of Health has stated that 60 – 80 % of population is suffering from dental caries and toothache is the 6th most common diseases in Indonesian society. So this is a challenge for dental profession to lower the rate of dental caries. The evidence is overwhelming that *Streptococcus mutans* is the primary etiological agent in the dental caries by triggering dental plaque formation. For this reason early prevention is needed by controlling dental caries in the community has been done in many ways, such as by tooth brushing regularly using dentifrice, mouth rinsing with antiseptics, and adding fluoride to toothpaste and drinking water in certain community. But actually dental caries still showed a high figure. This condition is a challenge for dentistry profession to prevent the dental caries and periodontal disease.. In this study is still needed further research to determine antibacterial property of infusum in tea leaves in controlling of dental caries.
It need to be done so the community as the consumers can consume medically accountable food and drinks.

The objective of research

The objective of this research is determining the sensitivity of infusum “Gopek” tea leaves by measuring:
- The inhibitory zone
- Minimum Inhibitory Concentration (MIC)
- Minimum Bactericidal Concentration (MBC) on:
  - *Streptococcus sobrinus* B13
  - *Streptococcus mutans* Ing-Britt
  - *Streptococcus mutans* LM7
  - *Streptococcus mutans* KPSK2, in vitro.

The Purpose and The Benefit of Research

By knowing its effectiveness, infusum tea can be used for mouth rinsing so it can inhibit the growth of mutans streptococci, the main bacteria that cause dental caries. This will hopefully use to keep the health of oral cavity and teeth especially by using in inexpensive way.

The method of Research

The material is used in this research: Gopek tea Infusum from Black tea leaves of *Camellia sinensis*.

The bacteria used as analysis unit are:
- *Streptococcus sobrinus* B13
- *Streptococcus mutans* Ing-Britt
- *Streptococcus mutans* LM7
- *Streptococcus mutans* KPSK2

In this case, those mutans streptococci are cultivated in TYS20B, BHI (Brain Heart Infusion Broth) and diagnostic sensitivity test (DST) was performed. Those specimen are incubated in anaerobic jar at 37°Celsius degree for 3 X 24 hours.

Working method

Sensitivity test to antibiotic can be done in two ways:

I. Drug serial dilution method:

a. Making the bacteria culture medium
   a.1. From the cultivated mutans streptococci in TYS20B, take one loop of bacteria and cultivate them in liquid culture medium BHI, then incubated it in anaerobic jar at 37 Celsius degree for 2 X 24 hours.
   a.2. After 2 days, compare the turbidity of bacteria media culture of BHI with Brown III standard solution.
   a.3. If it is found that bacteria culture in BHI media is more turbid, add sterile saline solution, little by little until the turbidity is equal to Brown III standard solution.
   a.4. If the turbidity of bacteria culture medium is equal to Brown III standard solution, the number of bacteria cell/ml in bacteria culture medium can be counted, their number is $\pm 9 \times 10^8$ bacteria /ml.
b. Bacteria dilution

If equalization process has been done, culture of mutans streptococci bacteria shall be diluted as follows:

b.1. Prepare 7 tubes of each 9 ml containing physiologic saline solution, and also prepare 1 tube containing 5 ml physiologic saline solution.

b.2. Take 1 ml bacteria from item a.4, the put into the first tube, shake them thoroughly from the first tube take 1ml bacteria culture and put into the second tube, and do the same thing through the seventh tube.

b.3. Take 5 ml of bacteria from the seventh tube and put them into the eighth tube and shake it thoroughly.

b.4. The number of bacteria is estimated ± 50 cells/ml each tube.

c. Sensitivity test of bacteria to Black tea Infusum.

c.1. Prepare 5 sets of test tubes, each tube is filled with 9 ml BHI and is labeled 1 – 5 respectively.

c.2. Put 1 gram of Black tea Infusum as much with 1:1 concentration into first tube then stir it well.

c.3. From the first tube, take 1 ml of solution and put into the second tube, do the same thing through the fifth tube.

c.4. After dilution finished, then put 1 ml of diluted *Streptococcus mutans* from item b.4 in those five test tube. All test tubes put in anaerobic jar at 37 Celsius degree for 2 X 24 hours.

C.5. After 72 hours, macroscopically we can see in which tube, the bacteria can not growth. Record the result to determine the Minimum Inhibition Concentration (MIC)

II. Method using disk with drug in solid media

II.1 Diluted 1 ml of mutans streptococci in the agar DST petri disc, the bacteria suspension wet the DST agar thoroughly.

II.2. Then put dilution of Black tea Infusum on a disk and put it on the surface of DST agar.

II.3. Those petri discs are incubated in anaerobic jar at 37 Celsius degree for 3 X 24 hours.

II.4. Inhibition zone will show around the disc and measuring the diameter of the isolated zone around the samples.

Data obtained is analyzed in a descriptive.
Results of Research

Table 1 showed that the Minimum Inhibitory Concentration (MIC) is 6.25%/ml and Minimum Bactericidal Concentration (MBC) 50%/ml for *Streptococcus mutans Ing-Britt*, *Streptococcus mutans LM7*, *Streptococcus mutans KPSK2* and *Streptococcus sobrinus B13*.

Table 1. Results of sensitivity test of mutans streptococci to “Gopek” Tea Infusum from Black tea leaves

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>The concentration of ‘Gopek’ Tea Infusum (%/ml)</th>
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<tbody>
<tr>
<td></td>
<td>50% (I)</td>
</tr>
<tr>
<td><em>S. mutans Ing-Britt</em></td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans KPSK2</em></td>
<td>-</td>
</tr>
<tr>
<td><em>S. mutans LM7</em></td>
<td>-</td>
</tr>
<tr>
<td><em>S. sobrinus B13</em></td>
<td>-</td>
</tr>
</tbody>
</table>

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

Table 2 The measurement of inhibitory zone which is carried out from the border of disc to zone with bacterial growth showed that inhibitory zone of *Streptococcus mutans Ing-Britt*, *Streptococcus mutans LM7*, *Streptococcus mutans KPSK2* and *Streptococcus sobrinus B13*.

Table 2. The result on inhibitory zone 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>S. mutans Ing-Britt</em></td>
<td>6.75</td>
</tr>
<tr>
<td><em>S. mutans KPSK2</em></td>
<td>1.40</td>
</tr>
<tr>
<td><em>S. mutans LM7</em></td>
<td>4.30</td>
</tr>
<tr>
<td><em>S. sobrinus B13</em></td>
<td>4.50</td>
</tr>
</tbody>
</table>

Discussion

The results show that standard strains of *Streptococcus sobrinus B13*, *Streptococcus mutans Ing-Britt*, *Streptococcus mutans LM7* and *Streptococcus mutans KPSK2* are have MIC at concentration of 6.25%/ml and MBC at concentration of 50%/ml Gopek tea infusum.

Inhibitory zone of *Streptococcus sobrinus B13* is 4.5 mm at the concentration 50% Gopek tea infusum.
Inhibitory zone of *Streptococcus mutans* Ing-Britt is 6.75 mm at the concentration 50 % Gopek tea infusum
Inhibitory zone of *Streptococcus mutans* LM7 is 4.3 mm at the concentration 50 % Gopek tea infusum
Inhibitory zone of *Streptococcus mutans* KPSK2 is 1.4 mm at the concentration 50 % Gopek tea infusum

**Conclusion and Suggestion**

From the research, it can be concluded that “Gopek” tea infusum from black tea leaves *Camellia sinensis* can inhibit the growth of Standard strains of mutans streptococci, in vitro.

Because of its antiseptic property, it also can be used as mouth wash to reduce the population of bacteria in oral cavity, so early infection can be anticipated.

**References**

4. Heriandi S. *Caries Activity on Schoolchildren in Jakarta*. J dent University of Indonesia 1993;(1);15-19

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