The Effect of *Fragaria x ananassa* Infusum on Salivary Mutans Streptococci

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**Abstract**

*Fragaria x ananassa* of strawberry fruit is known has an excellent source of vitamin C and flavonoid. One of the benefits of its active substance is the capability to inhibit the growth of oral bacteria. **Objectives:** The aim of this research is determining the sensitivity of infusum *Fragaria x ananassa* of strawberry fruit on mutans streptococci, *in vitro*. **Methods:** Infusum is the product of the process of steeping *Fragaria xananaassa* of strawberry fruit for extraction of its medicinal principles. The effect of infusum *Fragaria x ananassa* was examined by measuring the inhibition zone (agar diffusion method), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The microorganisms tested were composed 6 strains of mutans of *Streptococcus mutans* isolated from human’s saliva in Bangka Island Indonesia. Data obtained was done in a descriptive method. Results showed that *Fragaria x ananassa* infusum had effect on all of mutans of *Streptococcus mutans*1 (inhibition zone 0.30 mm; MIC 45%/ml, MBC 90%/ml); *Streptococcus mutans* 2 (inhibition zone 0.30 mm; MIC 45%/ml, MBC 90%/ml); *Streptococcus mutans* 3 (inhibition zone 1.10 mm; MIC 45%/ml, MBC 90%/ml); *Streptococcus mutans* 4 (inhibition zone 2.00 mm; MIC 45%/ml ,MBC90%/ml); *Streptococcus mutans* 5 (inhibition zone 1.20 mm; MIC 45% /ml, MBC90% /ml), *Streptococcus mutans* 6 (inhibition zone 1.90 mm; MIC 45%/ml ,MBC90% /ml) and their MBC value was two times bigger than MIC. **Conclusion:** We concluded that *Fragaria xananassa* Infusum has antimicrobial activity against mutans streptococci *in vitro*. Hence, it may have potential anti-caries property.

**Key words** *Fragaria x ananassa* –Infusum- Salivary Mutans Streptococci

**Introduction**

The Indonesian government has done some efforts to solve the dental health problems. As in reducing the dental caries prevalence, the government has done some kind of dental health education programs about brushing teeth properly and using mouthwash containing fluoride. Even though many efforts have been done by the government, the dental caries prevalence is still high.

Based on the problem above, as a dental profession I would like to make a contribution in reducing the high dental caries prevalence in Indonesia. Therefore I did a research about strawberry fruit and its effect to the dental caries.

Dental caries is the most common health problem in dental health. Dental caries is the progressive loss of tooth mineral followed by invasion into the demineralized tooth1. Dental caries is a relatively complex disease because it is not only damages the tooth surface, but also can be the focal infection of another disease in other parts of the body.

There are several bacteria that involve in dental caries process, but the most primary causative agent is *Streptococcus mutans* which cause dental caries by triggering the formation of dental plaque2-3...
One of the active components contained in the strawberry fruit is polyphenol compound, in great quantities. In this study is still needed further research to determine antibacterial property of infusum in strawberry fruit 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 the Study**

The aim of this research is determining the sensitivity of infusum *Fragaria x ananassa* of strawberry fruit on mutans streptococci, in vitro.

The effect of infusum *Fragaria x ananassa* was examined by measuring the inhibition zone (agar diffusion method), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

**Material and Methods**

The material used in this study is *Fragaria xananassa* of Strawberry on mutans streptococci, was examined in vitro.

The microorganisms tested were composed 6 strains of *Streptococcus mutans* isolated mutans isolated from human’s saliva in Bangka Island Indonesia labeled as S.mutans1, S.mutans2, S.mutans3, S.mutans4, S.mutans5, mutans6.

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°C Celsius degree for 3 X 24 hours

**Working Method**

- 100 gram Strawberry fruit is weighed, then mixed it with 500 ml of sterile aquadest in infusum pan and heated it on the gas stove. After the temperature has reached 900°C, keep it still on the gas stove and stir it once in a while.
- While it is still hot, pour it into a 500 ml Erlenmeyer gourd using a glass funnel, which has been lined with filter paper and muslin, until the volume reach 500 ml. From this process, 10% concentration of infusum tea solution is obtained.
- Put the Erlenmeyer gourd into water-bath, which contains boiled water keep it until the volume becomes 50 ml From this process 100% concentration of infusum tea solution is obtained.
- Keep the solution until the temperature decreases. Sterilization of tea Infusum done by using Tyndalisation method at temperature of 95°C Celsius degree for 30 minutes.

**Sensitivity Test**

Sensitivity Test to antibiotic can be done in two ways:

- Do the test in the laboratory by using the agar diffusion method to determine the inhibition zone.
- Do the test in the laboratory by using the broth dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).
A. Drug serial dilution method

Making the Bacteria Culture Medium

1. A portion of the strain of Mutans Streptococci in TYS20B media is taken to be cultivated in BHI agar. Then, put the cultivated bacteria inside an incubator at 37°Celsius degree for 48 - 72 hours in anaerobic condition.
2. After 2- 4 days, add the cultivated Mutans Streptococci, little by little into a physiologic salt solution until the turbidity is equal to McFarland I standard solution.
3. As the turbidity of culture bacteria medium has been equal to McFarland I standard solution, the number bacteria cells/milliliter can be counted their number is 0.3 X 10⁹ bacteria/milliliter.

Bacteria dilution

If equalization process has been done culture of Mutans Streptococci, shall be diluted as follows:

1. Prepare 7 tubes each containing 9 ml physiologic saline solution and prepare 1 tube containing 5 ml physiologic saline solution.
2. Take 1 ml bacteria from item a.1.3 then dilute culture medium bacteria until 10,000,000 times.
3. The bacteria dilution needs 7 times until the number of bacteria in the last tube is estimated at 30 cells/ml. Each tube shall be labeled.

Sensitivity test of bacteria to Strawberry fruit infusum

1. Prepare 8 tubes, each of the tubes is filled with 2 ml of Brain Heart Infusion Broth(BHI) agar. Each tube is labeled 1-8.
2. Add 2 ml of Strawberry infusum into the first tube, mix them until homogeneous.
3. Take 2 ml from the first tube, add it into the second tube, mix them until homogeneous. Do the same thing through the 8th tube.
4. After dilution is finished, then put 1 ml of the diluted bacteria from item a.2.3 in those eight test tube. All test tubes put in anaerobic jar at 37°Celsius degree for 2 X 24 hours.
5. 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 disc with drug in solid media

1. Pour dilute bacteria into the Diagnostic Sensitivity Test (DST) agar petri disc, the bacteria suspension shall wet the DST agar thoroughly.
2. Then put Strawberry infusum on the surface of a disc and it put it on the surface of DST agar.
3. Those petri discs are incubated in anaerobic jar at 37°Celsius degree for 3 X 24 hours.
Inhibition zone will appear around the disc and it shall be measured i.e. the diameter of the bacteria zone around the samples.

**Results**

The results of sensitivity test of Mutans Streptococci to Strawberry infusum with serial dilution method can be seen on the table 1 and the inhibitory zone can be seen on table 2.

Table 1. Result of sensitivity test of Mutans of *Streptococcus mutans* to Strawberry infusum

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>The concentration of Strawberry infusum(%/ml)</th>
<th>C +</th>
<th>C -</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90%</td>
<td>45%</td>
<td>12.5%</td>
</tr>
<tr>
<td>S.mutans1</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>S.mutans2</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>S. mutans3</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>S.mutans4</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>S.mutans5</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>S.mutans6</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>


table 1

(+) Growing C(+) Positive control without infusum Strawberry
(-) Not growing C(-) Negative control with infusum Strawberry

From the table above it can be seen that the minimum inhibitory concentration MIC is 45% and the minimum bactericidal concentration is 90% (MBC)

Table 2. 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>0.30</td>
</tr>
<tr>
<td><em>Streptococcus mutans2</em></td>
<td>0.30</td>
</tr>
<tr>
<td><em>Streptococcus mutans3</em></td>
<td>1.10</td>
</tr>
<tr>
<td><em>Streptococcus mutans4</em></td>
<td>2.00</td>
</tr>
<tr>
<td><em>Streptococcus mutans5</em></td>
<td>1.20</td>
</tr>
<tr>
<td><em>Streptococcus mutans6</em></td>
<td>1.90</td>
</tr>
</tbody>
</table>

The average zone of inhibition is 1.16 mm

**Discussion**

From the results showed that all of laboratory strains of *Streptococcus mutans1*, *Streptococcus mutans2*, *Streptococcus mutans3*, *Streptococcus mutans4*, *Streptococcus mutans5* and *Streptococcus mutans6* are sensitive to concentration 90%/ml Strawberry infusum.

Inhibitory zone of *Streptococcus mutans1* is 0.30mm; 0.30 mm for *Streptococcus mutans2*; 1.10 mm for *Streptococcus mutans3*; 2.00 mm for *Streptococcus mutans4*; 1.20 mm for *Streptococcus mutans5*; 1.90 mm for *Streptococcus mutans6*. 
Therefore Strawberry fruit infusum can be accepted inductively as a active substance that can inhibit the growth of mutans of *Streptococcus mutans*, in vitro.

**Conclusion and Suggestion**

From the study, it can be concluded that Strawberry fruit infusum can inhibit the growth of mutans of *Streptococcus mutans*. Strawberry fruit is traditional food from long time ago, but some people also use Strawberry fruit as traditional medicine to cure certain diseases. Because of its antiseptic property, a long term consuming Strawberry fruit are able to reduce the population of bacteria in oral cavity, so early infection can be anticipated.

**References**

5. Scott & Bailey: Diagnostic bacteriology. 5”th ed Mosby Co. 1958; 46-8

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