ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF GREEN TEA (CAMELLIA SINE NIS L.) AND ITS TOOTHPASTE PRODUCTS AGAINST STREPTOCOCCUS MUTANS AND LACTOBACILLUS ACIDOPHILUS

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Key words: Camellia sinensis, Antibacteria, S. mutans, Lactobacillus, Ethanolic extract, Toothpaste

Abstract: Streptococcus mutans and Lactobacillus acidophilus are bacteria caused dental caries in children. The aim of this research is to know the activity of ethanolic extract of green tea and its toothpaste product against S. mutans and Lactobacillus. One of green tea product from Indonesia was used as sample and was extracted by maceration method using 70% and 90% ethanol, respectively. Toothpaste products were making gel and paste dosageform. Antibacterial activity was tested by diffusion agar method using Muller Hinton Agar for S. mutans and Mann Rogosa Sharpe Agar for Lactobacillus. The results showed that 98% ethanolic extract of green tea had total polyphenol and antibacterial activity better than 79% ethanolic extract. Toothpaste content 5% extract of green tea had antibacterial activity against S. mutans more than Lactobacillus.

INTRODUCTION

Dental caries is an world public health challenge, especially among young children. 21% of children ages 6 to 11 year have had dental caries in their permanent teeth and 42% of children ages 2 to 11 year have had dental caries in their primary teeth (Anonim, 2015 and Colak et al., 2013).

There have been several publication on natural plants enriched polyphenol and flavonoid for preventing dental caries with antibacterial activity against Streptococcus mutans (Lucida et al., 2010; Osawae et al., 2001; Tahir and Moeen, 2011). S. mutans and S. sobrinus are the main cariogenic bacteria (Nurelhuwa et al., 2010).

Green tea extract is derived from the leaves of the plant Camellia sinensis L. Polyphenol green tea contains flavan-3-ol (catechin) and their derivatives including epicatechin (EC), gallocatechin (GC), (EGC), catechin gallate (CG), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG) and epigallocatechin gallate (EGCG) (Arita et al., 2015; Chu et al., 2003; Taylor et al., 2005).

Alcoholic solvents have been commonly used to extract polyphenols from natural sources. Addition of small amounts of water to organic solvents creates a more polar medium which facilitates phenolic extraction (Grujic et al., 2012).

For application green tea extract in preventing dental caries, we can make it in toothpaste. Toothpaste is a paste or gel dentifrice used with a toothbrush. Paste content abrasive agent, but gel is not.

The research are to know the activity of ethanolic extract of green tea and its toothpaste product against S. mutans and L. acidophilus.

MATERIALS AND METHODS

Green tea took from one of Indonesian product, ethanol (technical grade), demineral water, Folin Ciocalteaus reagent (Merck), Catechin (Sigma), Gallic acid (Sigma), Muller Hinton Agar (Merck), Mann Rogosa Sharpe Agar (Merck), paper disc
(Difco), Spectrophotometer UV-Vis (Agilent), St.
mutans and L. acidophilus (ATCC 27958, respectively).
Dimethylsulfoxide (DMSO), bases of toothpaste
(CaCO3, ethanol, hydroxyethylcellulose, sorbitol,
glycerine, sodium laurylsulphate, etc).

Preparation green tea extract

Each 100 g green tea powder were extracted by
remaceration method using 500 mL 70% ethanol and
90% ethanol, respectively, for 3 days in room
temperature. The ethanol was evaporated on rotary
evaporator and air dried until ethanol free - extract.

Analysis of total polyphenol and flavonoid in
green tea extract

Total polyphenol content in the ethanolic green tea
extracts of C. sinensis was determined by the Folin
Ciocalteau colorimetric method, gallic acid was
used as a standard. Total polyphenol content was
expressed as milligram of gallic acid equivalents
(GAE)/100 mg of samples (%). Total flavonoid was
determined by AlCl3 colorimetric method using
catechin as standard. Total flavonoid content was
expressed as milligram of catechin equivalents
(CAE)/100 mg of samples (%).

Antibacterial activity of green tea extracts

Antibacterial activity was done by diffusion agar
methods. The extract solution was made 1%, 3%,
5%, and 7% w/v using DMSO as solvent. 10 mL of
each culture was inoculated in 10 mL MH agar for
S. mutans and MRS Agar for L. acidophilus. Paperdisc
(6 mm in diameter) were placed on the media to
load each 10 mL of 1%, 3%, 5%, and 7% w/v extract
solution (100 µg/disc, 300 µg/disc, 500 µg/disc, and
700 µg/disc), respectively. The plates were incubated
at 37°C for 24 h. After 24 h, antibacterial activity of
the extract against both the test bacteria was observed
by and inhibition diameters were measured.

Formulation green tea extract toothpaste

Two formula toothpaste was made which containing
5% green tea extract in gel base (glycerin, sorbitol,
ethanol, sodium lauryl-sulphate, hydroxyethylcellulose, and sweetening and
flavouring agent), Formula A enriched with 30 %
CaCO3 as abrasive and Formula B without CaCO3.

Antibacterial activity of toothpaste

Antibacterial activity was done by diffusion agar
methods similarly in the extracts, with slightly
modification, like using well plates as sample
reservoir. The toothpaste was diluted 1:1 with
water. Formulation toothpaste was use as sample.

RESULTS AND DISCUSSION

Concentration ethanol as extracting solvent affected
in yields of extract, total polyphenol, and total
flavonoid. The yield of green tea extracted with 70% and
90% ethanol were 59.96 and 43.62% w/w,
respectively. As shown in Table 1 and after statistical
analysis by factorial design, content of flavonoid and
polyphenol in green tea extract was influenced
concentration ethanol as extracting solvent (p<0.05).
Polyphenol concentration in 90 % ethanolic extract
greater than 70% ethanolic, but flavonoid concentration in 90 % ethanolic extract smaller than
70 % ethanolic extract. There may be caused 70% ethanol more polar than 90 % ethanol. Green tea
contained polyphenol as flavonoid (catechin, epicatechin, epigallocatechin) and hydrozable tannin (epigallocatechin gallat, epicatechin gallat, galloclatechin gallat) with various solubility in
ethanol (Anita et al., 2015 and Roman et al., 2013),
ydrozable tannin more soluble in water/hidroalcohol than flavonoid.

Table 1. Concentration polyphenol and Flavonoid total
in Ethanolic green tea extract (% w/w)

<table>
<thead>
<tr>
<th>Extraction with ethanol</th>
<th>Concentration in extract (%)</th>
<th>Polyphenol total</th>
<th>Flavonoid total</th>
</tr>
</thead>
<tbody>
<tr>
<td>70%</td>
<td>21.36 ± 0.59</td>
<td>23.90 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>90%</td>
<td>23.44 ± 0.47</td>
<td>12.06 ± 0.22</td>
<td></td>
</tr>
</tbody>
</table>

% concentration in extract, expressed as
Mean ± SD (n = 3)

In Table 2 and 3 showed that ethanolic extracts of
green tea could inhibited growth of bacteria test. After
that, by factorial design, antibacterial activity of green tea extract was influenced
concentration ethanol as extracting solvent (p<0.05).
Green tea was extracted by 90% ethanol more
more effective than green tea extracted with 70% ethanol.
There are correlated in polyphenol content in green
tea extract. Diameter of zone inhibition of the 90 %
ethanolic extract in concentration 1 - 7 % (100 - 700
µg/disc) were 14.24 - 15.92 mm. The reseach antibacterial activity of Anita et al., (2015) showed
diameter of zone inhibition their ethanolic extract
in concentration 100 - 300 mg/disc were 10 - 18.33
mm against S. mutans and 8.33–12.67 mm against L.
Table 2. Diameter of inhibition zone (mm) green tea extract against S. mutans

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>100</th>
<th>300</th>
<th>500</th>
<th>700</th>
</tr>
</thead>
<tbody>
<tr>
<td>70%</td>
<td>14.36±0.79</td>
<td>14.16±0.56</td>
<td>14.27±0.59</td>
<td>15.14±0.69</td>
</tr>
<tr>
<td>90%</td>
<td>14.24±0.21</td>
<td>15.12±0.11</td>
<td>15.28±0.17</td>
<td>15.29±0.17</td>
</tr>
</tbody>
</table>

Table 3. Diameter of inhibition zone (mm) green tea extract against L. acidophilus

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>100</th>
<th>300</th>
<th>500</th>
<th>700</th>
</tr>
</thead>
<tbody>
<tr>
<td>70%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>90%</td>
<td>0</td>
<td>8.82±0.34</td>
<td>9.91±0.36</td>
<td>11.33±0.22</td>
</tr>
</tbody>
</table>

Inhibition zone in mm including disc, expressed as Mean ± SD (n = 3)

Table 4. Diameter of inhibition zone (mm) green tea extract toothpaste against S. mutans and L. acidophilus

<table>
<thead>
<tr>
<th>Bacterial test</th>
<th>Toothpaste samples</th>
<th>Paste of green tea extract</th>
<th>Gel of green tea extract</th>
<th>Herbal Paste Patent</th>
<th>Base toothpaste</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mutans</td>
<td>17.61</td>
<td>17.90</td>
<td>25.22</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L. acidophilus</td>
<td>16.34</td>
<td>16.45</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Diameter of Inhibition zone in mm including disc, expressed as Mean ± SD (n = 3)

acidophilus, respectively. Many factor affected zone inhibition of green tea extract, e.g. the variation in geographic location of plant specimen like soil and climate, extraction method, extraction solvent, and ratio samples-solvent, and antibacterial assay method. In this research were found that antibacterial activity green tea extract against S. mutans better than L. acidophilus. Tahir and Moen (2011) also found that ethanol extract of the leaves of C. sinensis possess greater antibacterial properties against L. acidophilus.

In Table 4, there was difference antibacterial activity between green tea and toothpaste.

Effect addition CaCO3 as abrasive did not affect in antibacterial activity. Comparing with herbal paste patent containing combination green tea extract and Piper betle leaves extract. It had antibacterial activity against S. mutans greater than the green tea toothpaste, but non antibacterial against L. acidophilus. Factors that affected, e.g. composition base toothpaste and concentration and various antibacterial compound.

CONCLUSION

90% ethanolic extract of green tea had total polyphenol and antibacterial activity better than 70% ethanolic extract. Toothpaste content 5% extract of green tea had antibacterial activity against S. mutans more than L. acidophilus and could be made toothpaste dosage form.

ACKNOWLEDGEMENT

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