Effectiveness of Roselle Effervescent Tablets as Traditional Medicinal Plants in preventing Growth of Candida albicans Colonies and Streptococcus mutans

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ABSTRACT

Aim: To analyze the effectiveness of roselle effervescent tablets inhibiting the growth of Streptococcus mutans and Candida albicans colonies on acrylic resin plate.

Materials and methods: A total of 90 plates of acrylic resin with S. mutans and C. albicans suspensions were divided into three groups of immersion: The first group was soaked in a solution of roselle effervescent tablets 10%, the second group soaked in solution of sodium perborate effervescent tablets, and the third group soaked in a solution of pure effervescent tablets each with an interval of 5, 10, and 15 minutes. The number of colonies of S. mutans and C. albicans was calculated before and after immersion to determine the effectiveness of effervescent tablets. Data were analyzed using t-test and one-way analysis of variance (ANOVA).

Results: The roselle effervescent tablets 10% showed a significant difference before and after immersion, thus inhibiting the growth of S. mutans and C. albicans colonies (p < 0.05). Roselle effervescent tablets 10% in immersion time 5, 10, and 15 minutes did not show any significant difference. The effectiveness of roselle effervescent tablets 10% did not show any significant differences with sodium perborate effervescent tablets.

Conclusion: Roselle effervescent tablets are effective in preventing S. mutans and C. albicans colonies like effectiveness with sodium perborate effervescent tablets.

Clinical significance: Roselle effervescent tablets 10% are effective in inhibiting the S. mutans and C. albicans colonies with immersion time of 5 minutes and have the same effectiveness with sodium perborate effervescent tablets.

Keywords: Candida albicans, Roselle effervescent tablets, Streptococcus mutans.
bacterial contamination. Denture cleansers can use either mechanical or chemical methods. Cleaning by chemical method consists of immersion of the denture in disinfectant solution and mechanical method by brushing or ultrasonic devices. Cleaning acrylic resin denture chemically is more effective than mechanical. So it needs cleaning materials that have a bactericide and fungicide, is easy to use, and is compatible with material denture.

To inhibit plaque formation on denture base in the field of dentistry, research is to be conducted using disinfectant solution packaged in effervescent tablet form with H. sabdariffa L. (roselle) flower extract. Effervescence is a reaction between citric acid compound with alkaline carbonate or bicarbonate. When dissolved in water, there is emission of gas, thereby allowing mechanical cleaning with gas bubbles as well as chemical cleaning by citric acid and other substances. In addition, the use of effervescent tablet is considered more convenient because it does not require a large container to store, with size and appropriate dosage.

Based on this background, this study was conducted to analyze the effectiveness and determine immersion time of the use of roselle effervescent tablets in inhibiting the formation of C. albicans and S. mutans colonies on acrylic resin plate.

MATERIALS AND METHODS

This study is an experimental research study (in situ) conducted at the Laboratory of Microbiology, University of Airlangga, using 10% roselle effervescent tablet, sodium perborate effervescent tablet found in the market (positive control), and pure effervescent tablet without the active substance of sodium perborate or roselle (negative control) with immersion time of 5, 10, and 15 minutes. The sample used is acrylic resin plate made of heat curing acrylic material with a size of 65 × 10 × 2.5 mm made in Asia Africa Laboratory, Bandung, with as many as 90 plates.

Manufacturing of 10% Roselle Extract

One hundred grams of dried roselle flower petals was mixed with hot water at 80°C which was then filtered, forming 10% roselle filtrate. Roselle filtrate was mixed with 50% dextrin and dried under 60°C vacuum for 8 hours, which resulted in 10% roselle extract.

Manufacturing of 10% Roselle Effervescent Tablet

Roselle effervescent tablet is made by mixing 10% roselle extract with 16% citric acid and 16% sodium bicarbonate for 2 minutes and then sieving 60 meshes and making the tablets.

Measurement of S. mutans and C. albicans

The number of S. mutans and C. albicans colonies was measured before and after immersing in effervescent tablet on 65 × 10 × 2.5 mm acrylic resin plate with the following procedures: Acrylic resin plate that has been sterilized in an autoclave at 121°C is inserted into the suspension of S. mutans or C. albicans and incubated for 24 hours at 37°C. The plates were rinsed with phosphate-buffered saline, then put in 10 mL brain heart infusion (BHI) broth or Sabouraud’s broth and vibrated with a vibrator for 30 seconds to produce a suspension of S. mutans and C. albicans. The suspension was then used using a 1 cc tuberculin syringe and incubated at tryptone, yeast extract, cystine (TYC) agar or Sabouraud’s dextrose agar for 48 hours at 37°C (pretest). The plates are then immersed in 10% roselle effervescent tablet solution for 5, 10, and 15 minutes and then put into 10 mL BHI broth or Sabouraud’s broth and vibrated with a vibrator for 30 seconds. The suspension was then taken using a 1 cc tuberculin syringe and incubated at TYC agar or Sabouraud’s dextrose agar for 48 hours at 37°C (posttest). Measurement of the effectiveness of S. mutans or C. albicans colonies was done by comparing the number of colonies pretest and posttest using a counter and expressed in colony-forming units/mL.

Data Analysis

This study using a one-way ANOVA test and Statistical Package for the Social Sciences version 20.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Effects of 10% roselle effervescent tablet solution immersion time against its effectiveness in inhibiting the number of S. mutans and C. albicans colonies on acrylic resin plate looks:

The results of the study (Table 1) showed no significant difference between the effectiveness of immersion times of 5, 10, and 15 minutes in the immersion group of 10% roselle effervescent tablet and sodium perborate effervescent tablet against its effectiveness in inhibiting the S. mutans and C. albicans colonies, whereas the immersing group with pure effervescent tablet showed significant differences in effectiveness between the immersion times of 5, 10, and 15 minutes.

DISCUSSION

This study used effervescent tablet made from extracts of roselle flower petals with a concentration of 10% of sodium perborate effervescent tablet which is in a package, and pure effervescent tablet without additional
Effectiveness of Roselle Effervescent Tablets as Traditional Medicinal Plants

Table 1: Difference of the effectiveness of immersion groups to the number of C. albicans and S. mutans colonies by the time of immersion

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Immersion group</th>
<th>5 minutes Mean ± SD</th>
<th>10 minutes Mean ± SD</th>
<th>15 minutes Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. mutans</td>
<td>10% roselle effervescent tablet</td>
<td>106.00 ± 1.00</td>
<td>106.40 ± 2.41</td>
<td>107.07 ± 2.40</td>
<td>0.135</td>
</tr>
<tr>
<td></td>
<td>Sodium perborate effervescent tablet</td>
<td>107.20 ± 1.30</td>
<td>107.80 ± 1.79</td>
<td>109.20 ± 1.30</td>
<td>0.133</td>
</tr>
<tr>
<td></td>
<td>Pure effervescent tablet (control)</td>
<td>64.00 ± 3.40</td>
<td>71.60 ± 2.97</td>
<td>80.00 ± 2.92</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>10% roselle effervescent tablet</td>
<td>102.60 ± 2.19</td>
<td>104.40 ± 2.51</td>
<td>104.60 ± 2.79</td>
<td>0.409</td>
</tr>
<tr>
<td>C. albicans</td>
<td>Sodium perborate effervescent tablet</td>
<td>104.60 ± 2.07</td>
<td>106.80 ± 1.79</td>
<td>106.80 ± 1.92</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td>Pure effervescent tablet (control)</td>
<td>45.00 ± 2.45</td>
<td>56.60 ± 3.58</td>
<td>65.60 ± 3.51</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Normality test, Shapiro–Wilk test: p > 0.05; normal distribution of data; *One way ANOVA test: p < 0.05; significant

effectiveness of antimicrobial with a vulnerable time of 4 to 8 minutes. This approaches immersion protocol of acrylic resin plate effervescent in a hydrogen peroxide solution is effective as immersion acrylic resin plate for 30 minutes. This approaches immersion protocol of effervescent dental cleanser at 50°C for 3 minutes twice a day. According to Lee the effervescent is resolved when dispersed well with time ≤5 minutes.16

This study showed after immersion of 10% roselle effervescent tablet, sodium perborate effervescent tablet and pure effervescent tablet without the active substance sodium and roselle, seen in inhibiting the number of S. mutans and C. albicans colonies on acrylic resin plate. This is supported by the research of Desrosiers et al.17 which states that some types of denture cleanser containing citric acid are chemotherapeutic agents that can effectively interfere with bacterial biofilm matrix by inhibiting calcium ions, resulting in antibiofilm activity. Ntrouka et al.18 study that assessed the ability of citric acid solution given on the surface of the implant showed a decrease in the number of pathogenic species, especially the species of S. mutans bacteria. Izumi et al.19 study revealed that denture cleansers using organic acids showed a decrease in the number of species of Streptococcus sanguinis, Streptococcus pneumoniae, C. albicans and their effects are strong as commercial denture cleaners and safe to use by old people. Faot et al.20 stated that denture cleanser citric acid has effect in inhibiting C. albicans after treatment but cannot eliminate the biofilm as a whole. Roselle effervescent tablets 10% proved to reduce the number of bacterial and fungal colonies due to the content of polyphenols. A study conducted by Sayago-ayerdi et al.21 showed that 66% of the polyphenol content contained in the roselle flower is polyphenol. Research conducted by Nurchasah et al.22 proved that the flavonoid contained in the plant roselle is one of many groups of active substances. Flavonoids work by denaturing proteins and increasing the permeability of cell membranes.23 The function of the disrupted cell membrane can cause disruption in cell formation, resulting in cellular damage to Candida and Streptococcus bacteria. The damage causes the death of C. albicans and Streptococcus bacteria, according to Jawetz study. Polyphenol compounds decrease bacterial activity so that it indirectly influences inhibition of activity of colony formation of bacteria and fungi on denture plate.25

CONCLUSION

Roselle effervescent tablets 10% are effective in inhibiting C. albicans and S. mutans colonies with immersion time of 5 minutes and have the same effectiveness with sodium perborate effervescent tablet.

REFERENCES