Effects of Differing Concentrations of Bilimbi (*Averrhoa bilimbi* L.) Extract Gel on Enamel Surface Roughness

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**Abstract.** Bilimbi (*Averrhoa bilimbi* L.) is an acidic extract used to bleach teeth. This study assessed the effect of two types of bilimbi extract (Bogor or Aceh) on enamel surface roughness. The bilimbi extracts were applied at concentrations of 70%, 80%, or 90% to bovine enamel specimens for 4 h per day for 14 days. Analysis showed that all groups had significant changes in surface roughness (*p* < 0.05) after 7 and 14 days of application. The group treated with 90% bilimbi showed the most significant change in surface roughness. The changes in enamel surface roughness were affected by the pH, concentration, and duration of exposure to bilimbi extract gel.

1. **Introduction**

Previous research has identified the plant bilimbi (*Averrhoa bilimbi* L.) as a suitable material for bleaching teeth [1]. Bilimbi consists of carboxylic compounds, in the form of oxalic acid, and peroxide compounds that are expected to bleach discolored teeth [1]. However, bilimbi is acidic with a pH of 0.9–1 [2]. Enamel demineralization, the dissolving process of hydroxyapatite (HA) crystals in tooth enamel, is known to occur at pH levels below 5.5. As a pH of 5.5 is the critical point for HA to dissolve [3], acidity levels are expected to affect enamel surface roughness. This effect was confirmed by Sungkat et al. [4] who showed that sour, fruity beverages increase surface roughness *in vitro*. Increased enamel surface roughness may cause plaque retention and maturation processes, resulting in an increased demineralization rate and, potentially, oral disease [5]. Although work by Fauziah et al. [1] confirmed that bilimbi is effective for bleaching teeth, further research is needed to determine a safe concentration of bilimbi for application to teeth without excessive demineralization.

The aim of this research is thus to test the effect of bilimbi extract formed into a gel with a concentration of 90%, 80%, or 70% on enamel surface roughness. Bilimbi gel was applied for 4 h per day for 14 days to represent home tooth bleaching techniques. Previous research used bilimbi obtained from Aceh. In this research, we test bilimbi from Aceh and Bogor.
2. Methods

This experimental study was performed at the Dental Materials Laboratory, Faculty of Dentistry, Universitas Indonesia, from September to December 2015. The sample consisted of 30 bovine incisives. The inclusion criteria were no caries, no anomalies, and a good labial surface. The sample size was determined by the Federer formula.

This experiment began by root cutting the 30 bovine incisives using dental burs to one-third of the way to the middle of the crown. The teeth were then painted with acrylic resin, and the labial surface was smoothed using a grinding machine with sandpaper no. 2000. The smoothing was performed with the provision that no more than 0.1 mm of enamel was removed (measured with calipers). Polishing was performed using a polishing machine with alumina 1 μ. The specimens were washed after the smoothing and polishing process, and the initial roughness of enamel labial surfaces to be treated with gel was measured using a surface roughness tester (Mitutoyo SJ 301). Each tooth was then placed in a plastic pot with a serial number, and the 30 specimens were randomly divided into six equal treatment groups.

Aceh and Bogor bilimbi extracts were prepared using the maceration method and stored at the Balitro Bogor laboratory. The pH and composition tests were performed by the Balitro Bogor Laboratory. The gels were made at the Pharmacy Department, Faculty of Medicine, University of Indonesia Laboratorium by mixing bilimbi extract with carboxymethyl cellulose to achieve three concentrations: 70%, 80%, and 90%.

The Aceh and Bogor bilimbi gels of varying concentrations were applied to the specimens using a brush. Specimens coated with bilimbi gel were placed in an incubator at 37 °C (to represent the temperature of the oral cavity) for 4 h. After 4 h, the specimens were removed from the incubator, cleaned, and sprayed with aquadest using syringe. The labial surface was gently wiped with absorbent paper, and the specimen was placed back in the plastic pot. This procedure was performed each day for 14 days. The surface roughness was measured after 7 and 14 days of application.

The resulting data were analyzed using SPSS 17.0 (IBM) software. Paired t-tests were used to assess the differences in roughness values before and after application. Unpaired t-tests were used to assess differences in surface roughness within the concentration groups.

3. Results

As shown in Table 1, there was an increase in enamel surface roughness after 7 days of application of the Bogor and Aceh bilimbi extract gels and a further increase after 14 days. The organic acid contents of the two bilimbi extracts were similar and included acetic acid, citric acid, and oxalic acid (Table 2). Citric acid was the main organic acid present in bilimbi extract. The pH values of the two bilimbi extracts were also similar, with the Bogor bilimbi extract having a pH of 1.75 and the Aceh bilimbi extract having a pH of 2.04.

| Experimenta l Group | Concentration | n | Average Ra ± SD |
|---------------------|---------------|---|-----------------|
|                     |               |   | Baseline        | Day 7   | Day 14          |
| Gel with            | 70%           | 5 | 0.154 ± 0.005   | 0.224 ± 0.009 | 0.404 ± 0.082   |
| Bilimbi extract from Bogor | 80% | 5 | 0.142 ± 0.004   | 0.24 ± 0.019  | 0.708 ± 0.117   |
|                     | 90%           | 5 | 0.134 ± 0.005   | 0.27 ± 0.01  | 1.724 ± 0.148   |
Table 2. Organic acid components of 100% Bogor and Aceh bilimbi extract.

| Sample Name            | Component | Result          |
|------------------------|-----------|-----------------|
|                        | Organic Acid: |               |
| Bogor Bilimbi Extract  | Acetate   | 730.79 ppm      |
|                        | Citrate   | 3526.91 ppm     |
|                        | Oxalate   | 188.96 ppm      |
|                        | Malate    | -               |
| Aceh Bilimbi Extract   | Acetate   | 565.95 ppm      |
|                        | Citrate   | 3551.67 ppm     |
|                        | Oxalate   | 135.01 ppm      |
|                        | Malate    | -               |

All specimen groups showed increased enamel surface roughness after 7 days of application, and the surface roughness further increased after 14 days. The changes in enamel surface roughness from initial levels were significantly different ($p < 0.05$) after 7 and 14 days for groups treated with Aceh and Bogor bilimbi extract gel at all concentrations (90%, 80%, and 70%).

The specimen groups treated with 90% bilimbi from Aceh or Bogor showed greater changes in enamel roughness after 7 and 14 days compared with the groups treated with 80% or 70% bilimbi gel extract. For the Bogor bilimbi group, paired t-tests indicated significant differences ($p < 0.05$) in surface roughness after 7 and 14 days of application with 90%, 80%, or 70% concentrations. For the Aceh bilimbi group, there was significant difference ($p < 0.05$) in surface roughness between the 90%, 80%, and 70% groups. However, there was no significant difference ($p > 0.05$) in surface roughness between specimens treated with 90% gel and 80% gel after 7 days. After 14 days of application, the changes in surface roughness were statistically significant ($p < 0.05$) in all concentration groups (90%, 80%, and 70%).

4. Discussion

In this research, specimens treated with Bogor and Aceh bilimbi extract at concentrations of 90%, 80%, and 70% all showed increased roughness. This is due to the high acidity of bilimbi, which was shown to contain oxalic and citric acid (Table 2). Research by Yurlı et al. [6] demonstrated that 0.1% citric acid could increase enamel surface roughness. Yanfang et al. [7] showed that exposure to acidic fruit juice may increase enamel surface roughness. This was confirmed by Birgül et al. [8] who showed that apple juice with a pH of 3.8 caused mineral loss in teeth and increased enamel surface roughness. Machado et al. [9] applied orange juice with a pH of 3.46 to enamel and reported a significant increase in surface roughness. It is thus likely that exposure to the acids in Aceh and Bogor bilimbi extract gel caused erosion of tooth enamel. The pH was not assessed for each concentration, but all of the bilimbi gels are expected to have a pH below 5.5 given the pH of 100% bilimbi.

A pH of 5.5 is a critical point for HA calcium to demineralize. The process of dissolving HA causes lesions in the form of white spots. White spots occur when the demineralization process is not counterbalanced by remineralization. In this research, we did not provide supporting materials that may initiate remineralization. The white appearance and optical phenomena were caused by minerals on the
surface and subsurface of the enamel. Dissolution of HA crystals on the enamel caused demineralization on the subsurface that created holes between the enamel rods, leading to enamel surface roughness [10]. We thus suspect that more exposed surfaces treated with bilimbi extract gel would result in increasing enamel surface roughness.

Specimens treated with 90% Bogor bilimbi gel showed greater changes after 7 and 14 days compared with specimens treated with 80% and 70% gel. A similar pattern was seen in specimens treated with Aceh bilimbi gel. These results indicate that the more bilimbi extract in the gel, the lower the pH and thus the greater increase in surface roughness. We also found that the average enamel surface roughness value after treatment with 70% bilimbi gel was more than 0.2 µm, whereas the value was more than 0.5 µm after treatment with 80% or 90% bilimbi extract gel. Higher roughness values could allow bacteria to attach more easily [11], which could further boost demineralization rates, and would be felt by the human tongue [12].

5. Conclusion
Bilimbi extract from Bogor and Aceh with concentrations of 90%, 80%, and 70% significantly influenced tooth enamel roughness surface after 7 to 14 days. Changes in surface roughness were influenced by the type of Bilimbi gel, the pH of the gel, and duration of application.

6. References
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