Effect of application of betel leaf extract gel on enamel surface roughness

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Abstract. This study was conducted to determine the effect of application of betel leaf extract gel at different concentrations on enamel surface roughness. We treated 18 bovine enamels with betel leaf extract gel at 15%, 25%, and 25% concentrations every 4 min for 20 min, 40 min, and 44 min. We found a decrease in surface roughness with the application of 15% and 35% betel leaf extract gel for 20 and 40 min and an increase after 44 min. With the application of 25% betel leaf extract gel, we found an increase after 20 min but a decrease after 40 and 44 min. We conclude that betel leaf extract gel affects the surface roughness of tooth enamel. The most effective application was with 15% betel leaf extract gel for 3 months.

1. Introduction
In Indonesia, many types of leaves are used as traditional herbs, including the betel leaf (*Piper betle*) [1]. As a tradition in Indonesia, betel leaves are chewed or consumed at religious events and are used as an antiseptic or pain relief material for toothache. Betel leaf extract is a proven antibacterial agent that can treat many conditions such as toothache, bleeding, and abscesses [2].

In dentistry, betel leaf extract has been proven to decrease bacterial and fungal activity in the oral environment. Hydroxychavicol in betel leaves is an effective antifungal for many types of fungi in the oral environment [3]. Betel leaf extract also contains catechol, which is derived from polyphenols and flavonoids, which are high in antioxidants [4]. A study conducted by Hasim (2003) showed that antimicrobial agents in betel leaves are higher than in NaF [5]. Additionally, a number of studies have showed that the antibacterial activity of betel leaves is higher than other herbs; hence, betel leaves are used in toothpaste and mouthwash products to prevent caries [6]. To the best of our knowledge, no other study has demonstrated the use of betel leaves in the form of gel.

Topical fluoride gel has longer exposure time than toothpaste and mouthwash and consequently affects enamel surface roughness. Surface roughness is an important factor because a rough enamel can be a place for bacterial adherence and colonization, thereby increasing demineralization and gingival infection [7]. Enamel surface roughness is an irregularity caused by friction, excessive use, and mechanical or chemical scraping [8]. Surface roughness is calculated based on vertical deviation and midline. A bigger deviation indicates a rougher enamel, and conversely, a lower deviation indicates a smoother enamel [9]. This study aimed to assess the effect of a topical betel leaf extract gel on enamel surface roughness and to quantify the effect of different concentrations of topical betel leaf extract gels.
2. Methods
Eighteen bovine teeth were cut between the crown and root using a grinding machine, and the unused roots were disposed. The specimens were cleaned and aligned with a wheeling sandpaper and sanded using a grinding machine and a sandpaper (#2000). Sanding was limited to 0.1 mm. Following sanding, the specimens were washed again. Sanding was performed to obtain a flat surface for surface roughness measurement. The surface roughness measurement value before and after gel application was conducted using the surface roughness tester Mitutoyo SJ301. Testing was conducted thrice to prevent miscalculations. The measurement results is Ra, the average enamel surface roughness value in µm.

The 18 specimens were obtained by Federer formula and divided into three groups of six samples each. Gels with different concentrations of betel leaf extract (15%, 25%, and 35%) were applied to each group. The gels were applied with an etching brush for 4 min. The specimens were then rinsed with running water before the gels were applied again for an additional 4 min. Applications were performed every 4 min for 104 min. The specimens were then evaluated to quantify the average enamel surface roughness after 20, 40, and 44 min, assuming that real-life measurements would be conducted at one, three, and six months. It was assumed that real-life gel application would be performed once a week for 4 min over a period of 6 months. We conducted the statistical analyses using one-way ANOVA and repeated ANOVA.

3. Results
Table 1 show that there is a difference in enamel surface roughness before and after application of betel leaf extract gel at concentrations of 15%, 25%, and 35% after one, three, and six months. In the 15% concentration group, enamel surface roughness decreased after gel application for 1 month, and it decreased again after 3 months of application. After 6 months of application, the average enamel surface roughness increased but was not higher than that average value before application. In the 25% concentration group, the average enamel surface roughness increased after 1 month of application, but roughness decreased after 3 months of application and decreased further after 6 months of application. In the 35% concentration group, the average enamel surface roughness decreased after 1 month of application, but roughness decreased after 3 months of application and decreased further after 6 months of application. After 6 months of application, the average surface roughness increased but was not higher than that before application.

| Betel leaf extract concentration (%) | Sample size | Enamel surface roughness (µm) |
|-------------------------------------|------------|-------------------------------|
|                                     |            | Before application | After 1 month | After 3 months | After 6 months |
| 15                                  | 6          | 0.207 ± 0.03          | 0.139 ± 0.02  | 0.126 ± 0.03  | 0.194 ± 0.03  |
| 25                                  | 6          | 0.195 ± 0.03          | 0.228 ± 0.06  | 0.148 ± 0.04  | 0.127 ± 0.03  |
| 35                                  | 6          | 0.207 ± 0.02          | 0.141 ± 0.03  | 0.136 ± 0.04  | 0.169 ± 0.03  |

In the 15% concentration group, the average enamel surface roughness decreased significantly (p < 0.05) after 1 and 3 months compared with the initial average, but the average values between 1 and 3 months did not significantly differ. The average value from 3 to 6 months increased significantly, although the value after 6 months did not significantly differ from the initial average value (Table 2).
Table 2. Repeated ANOVA of average enamel surface roughness before and after application of betel leaf extract gel at 15% concentration for one, three, and six months

| (I) time (J) time | Mean Difference (I − J) | Standard Error | P-value |
|------------------|-------------------------|----------------|---------|
| 1 2              | 0.068*                  | 0.015          | 0.005   |
| 3                | 0.081*                  | 0.012          | 0.001   |
| 4                | 0.012                   | 0.024          | 0.634   |
| 2 1              | −0.068*                 | 0.015          | 0.005   |
| 3                | 0.013                   | 0.012          | 0.318   |
| 4                | −0.056*                 | 0.013          | 0.008   |
| 3 1              | −0.081*                 | 0.012          | 0.001   |
| 2                | −0.013                  | 0.012          | 0.318   |
| 4                | −0.068*                 | 0.022          | 0.027   |
| 4 1              | −0.012                  | 0.024          | 0.634   |
| 2                | 0.056*                  | 0.013          | 0.008   |
| 3                | 0.068*                  | 0.022          | 0.027   |

P (sig.) < 0.05. 1) before application, 2) after 1-month application, 3) after 3-month application, 4) after 6-month application.

In the 25% concentration group, there was an insignificant increase (p > 0.05) after gel application after one month and then a significant decrease after gel application for 3 months, although the average values after 1 and 3 months did not significantly differ compared with those before application. The average enamel roughness after six months significantly decreased compared with that before application, but the value was not significantly different from that after three months (Table 3).

Table 3. Repeated ANOVA of average enamel surface roughness before and after application of betel leaf extract gel at 25% concentration for one, three, and six months

| (I) (J) | Mean difference (I − J) | Standard error | P-value |
|---------|-------------------------|----------------|---------|
| 1 2     | −0.033                  | 0.03           | 0.401   |
| 3       | 0.047                   | 0.02           | 0.082   |
| 4       | 0.068*                  | 0.01           | 0.001   |
| 2 1     | 0.033                   | 0.03           | 0.401   |
| 3       | 0.080*                  | 0.02           | 0.016   |
| 4       | 0.101*                  | 0.02           | 0.019   |
| 3 1     | −0.047                  | 0.02           | 0.082   |
| 2       | −0.080*                 | 0.02           | 0.016   |
| 4 1     | −0.068*                 | 0.01           | 0.001   |
| 2       | −0.101*                 | 0.02           | 0.019   |
| 3       | −0.021                  | 0.01           | 0.223   |

P (sig.) < 0.05. 1) before application, 2) after 1-month application, 3) after 3-month application, 4) after 6-month application.
In the 35% concentration group, there was a significant decrease in enamel surface roughness after gel application after 1 and 3 months, although the average values after 1 and 3 months did not significantly differ from each other. There was a significant increase after 6 months of application compared with that after three months of application, and the average enamel surface roughness value was not significantly different from that before application (Table 4).

Table 4. Repeated ANOVA of average enamel surface roughness before and after application of betel leaf extract gel at 35% concentration for 1, 3, and 6 months.

| (I) | (J) | Mean difference (I − J) | Standard error | P-value |
|-----|-----|-------------------------|----------------|---------|
| 1   | 2   | 0.067*                  | 0.016          | 0.010   |
| 3   |     | 0.071*                  | 0.020          | 0.016   |
| 4   |     | 0.038                   | 0.019          | 0.094   |
| 2   | 1   | −0.067*                 | 0.016          | 0.010   |
| 3   |     | 0.004                   | 0.014          | 0.761   |
| 4   |     | −0.028                  | 0.016          | 0.146   |
| 3   | 1   | −0.071*                 | 0.020          | 0.016   |
| 2   |     | −0.004                  | 0.014          | 0.761   |
| 4   |     | −0.033*                 | 0.012          | 0.040   |
| 4   | 1   | −0.038                  | 0.019          | 0.094   |
| 2   |     | 0.028                   | 0.016          | 0.146   |
| 3   |     | 0.033*                  | 0.012          | 0.040   |

P (sig.) < 0.05. 1) before application, 2) after 1-month application, 3) after 3-month application, 4) after 6-month application.

Comparing the average enamel surface roughness values after 1, 3, and 6 months of application with those before application, no values showed marked differences from each other. This study tested the data with a one-way ANOVA because the data were normal in a normality test. The variance in the data was found to be the same, and thus there were no significant differences in any group.

4. Discussion

This study examined the effect of application of betel leaf extract gel at 15%, 25%, and 35% concentrations on enamel surface roughness after 1, 3, and 6 months. Bacteria can increase demineralization by adhering to rough enamel surface with a 0.2-µm threshold [10]. The study conducted by Fujii et al. (2011) showed that enamel surface roughness is affected by factors such as pH of treatment materials, exposure period, and calcium and phosphate content in the materials [11]. Cochrane et al. (2009) stated that pH from treatment materials is the biggest determinant of enamel roughness [12]. A study by Fujii et al. (2011) proved that 1 min of exposure to a drink with low pH (pH 2.0–4.0) can cause an increase in the average enamel surface roughness, whereas drinks with neutral pH (pH 6.3) do not show significant differences after different exposure periods [11]. We conducted pH testing to prove that betel leaf extract gels do not have low pH at 15% (pH = 6.16), 25% (pH= 6.16), and 35% (pH= 6.24) concentrations. This is also supported by research conducted by Wang et al. (2012) that compared many types of pH on the enamel surface. He concluded that a neutral pH is not only increasing surface hardness but also prevented surface roughness [13].

In the 15% and 35% concentration groups, we found a decrease in the average enamel surface roughness after 1 and 3 months of application and an increase after 6 months. The decrease until 3 months of application is assumed to be caused by tannin deposition on the enamel surface. This is supported by a study by Yu et al. (1995) which found that when enamel surface is exposed to tannin, there were calcium increases as well as resistance to acid, which could prevent calcium from dissolving.
in acidic solutions [14,15]. Although more tannin is deposited with a longer application, dissolution from apatite may occur, causing the surface roughness to increase [16]. This is demonstrated by the increase in enamel surface roughness after 6-month application. Tannin content in betel leaf extract gel is displayed in Table 5.

| Betel leaves extract concentration (%) | Tannin content (%) |
|----------------------------------------|--------------------|
| 15                                     | 2                  |
| 25                                     | 4                  |
| 35                                     | 5                  |

Contrary to the 15% and 35% concentration groups, in the 25% concentration group, the average enamel surface roughness increased (although not significantly) after 1 month of application, but significantly decreased after 3 and 6 months of application. This transient increase is assumed to be caused by a transient decrease in enamel pH before returning to normal values. This finding is in accordance with a study conducted by Simon et al. (2001) that showed a decrease in enamel pH after exposure to drinks containing tannin. The decrease in surface pH is not only small but also short-lived; hence, pH returns to normal level in seconds or minutes. Fujii et al. (2011) also stated that there is a significant correlation between enamel surface roughness and pH level, this is indicating that surface roughness varies with surface pH after exposure to drinks, unrelated to the initial pH of the drink or the exposure period [11].

In all concentration groups, the one-way ANOVA showed no significant differences between average enamel surface roughness before gel application and after 1, 3, and 6 months of application. This result may be explained by the fact that the pH of betel leaf extract does not significantly differ among concentrations.

5. Conclusion

It is found that application of betel leaf extract gel for 1, 3, or 6 months can affect enamel surface roughness, whereas different concentrations of betel leaf extract do not cause significant changes in enamel surface roughness.

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