Effect of betel leaf extract gel on the hardness of enamel

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Abstract. The main component of betel leaf (Piper betle Linn.) is tannin, which easily binds with proteins and can affect enamel hardness. Betel leaf extract was applied as a topical gel to the enamel of 18 bovine teeth specimens embedded in acrylic resin (three groups of 15%, 25%, and 35% gel applied for 1, 3, and 6 months). Enamel hardness was measured with a Knoop microindentation tester. Statistical analysis was done by repeated and one-way analysis of variance tests. A significant increase in microhardness was noted after 6 months of 15% betel leaf extract application (P < 0.05). Betel leaf extract gel can significantly affect enamel hardness.

1. Introduction
A tradition of the ancient community is to suck betel leaves mixed with gambier and chalk. On the basis of research, sucking betel leaf can make teeth stronger. However, the habit has been gradually abandoned by the community, because of the current belief that sucking betel leaf is less clean and produces excessive saliva. Betel is a plant well known to the public and widely used in traditional medicines, such as those used for asthma, rheumatism, and wound healing [1]. Betel leaf also can help prevent oral disease. Antiseptic agents can inhibit the biological synthesis of Streptococcus mutans (the bacteria that causes dental caries) so that dental plaque does not cause caries. Polyphenols in betel leaf (Piper betle Linn.) act as antiseptic agents, which allow its use for inhibition of dental caries.

Caries is an oral disease with the highest prevalence in Indonesia [2] (90.5% according to the 2004 Household Health Survey [3]). Caries is caused by demineralization of enamel due to exposure to lactic acid produced by S. mutans [4,5]. Presently, there are many ways to prevent dental caries, such as regular brushing, gargling with antiseptic, and application of fluoride. Products also have been developed with betel leaf as an ingredient. The antiseptic function of betel leaf is 3–4 times better than that of fluoride and its use is promising. Currently, there are many product choices, including toothpaste and mouthwash, and the topical gel form has not been much developed. On the basis of the use of topical fluorides as a demineralizing inhibitor and for improving tooth remineralization [6], gel application has several advantages, such as longer exposure time. Topical fluoride has a long application time of 5 min with a 1 h interval after which the subject should not eat or drink [7], which is much longer compared with the time spent for application of mouthwash and toothpaste. Therefore, gel application products are expected to be an alternative in maintaining healthy teeth and mouth.
The enamel is the outermost layer and hardest structure on the teeth, consisting mostly of hydroxyapatite crystals [Ca_{10}(PO_4)_6(OH)_2]. These crystals are permeable to ions and molecules that are in direct contact with the surface. Acids contacting the enamel will cause dissolution and decreased hardness of the tooth surface, thus causing dental caries [8].

This study determined the effect on enamel hardness of certain concentrations of betel leaf extract gel applied for 1, 3, and 6 months.

2. Methods
This experimental study was done at the Dental Materials Laboratory, Faculty of Dentistry, Universitas Indonesia. The 18 specimens studied were divided into three treatment groups of six specimens each. Before treatment started, the specimens were prepared. In each group, betel leaf extract gel (15%, 25%, and 35%) was applied every 4 min for 104 min (assuming equivalent use for 4 min, once a week for 6 months). The initial hardness value was tested. Then, the gel was applied for the first 20 min (i.e., “1 month”), and hardness was tested again. The gel was reapplied for the next 40 min (“3 months”), and the hardness was tested again. Finally, the gel was reapplied for the last 44 min (“6 months”), and the hardness was retested. Following each application, the enamel hardness of each specimen was measured using the Knoop Microhardness Tester tool. The data were then analyzed using ANOVA and Post-hoc test.

3. Results
Table 1 shows the mean enamel hardness before and after application of 15%, 25%, and 35% betel leaf extract gel application for 1, 3, and 6 months. After application of 15% gel, mean enamel hardness decreased at 1 (295.8 ± 5.18 KHN) and 3 (212.2 ± 7.15 KHN) months, but increased (244.2 ± 8.50 KHN) after 6 months. Decreases in mean enamel hardness values at 1, 3, and 6 months were 378.5 ± 6.47, 314.2 ± 7.48, and 261.5 ± 7.15 KHN, respectively, for the 25% gel group, and 346.3 ± 4.89, 307.7 ± 7.21, and 268.8 ± 5.12 KHN, respectively, for the 35% gel group.

| Betel Leaf Extract Gel Concentration | N  | Mean enamel hardness (KHN) |
|-------------------------------------|----|---------------------------|
|                                     |    | Before Application | 1 month Application | 3 months Application | 6 months Application |
| 15%                                 | 6  | 403.8 ± 5.61       | 295.8 ± 5.18        | 212.2 ± 7.15        | 244.2 ± 8.50        |
| 25%                                 | 6  | 410.8 ± 12.21      | 378.5 ± 6.47        | 314.2 ± 7.48        | 261.5 ± 7.15        |
| 35%                                 | 6  | 400 ± 5.58         | 346.3 ± 4.89        | 307.7 ± 7.21        | 268.8 ± 5.12        |

On the basis of one-way analysis of variance (ANOVA) testing, we concluded that there was a significant difference between the 15%, 25%, and 35% concentration groups. Post hoc least significant difference analysis showed a significant difference in enamel hardness with application of 15% and 25% betel leaf extract gel after 1 month.

4. Discussion
Enamel hardness decreased after application of 15% betel leaf extract gel for 1 and 3 months and then increased after the next 6 months of application. Meanwhile, at concentrations of 25% and 35%, there was a statistically significant continuous decrease in hardness after 1, 3, and 6 months. This decrease in enamel hardness was allegedly related to the content of tannin in the betel leaf extract gel. Menuka et al. [9] reported
that *Piper betle* Linn. betel leaf extract contained phenol, tannin, flavonoid, and alkaloid compounds. Using spectrophotometric methods, Balitro reported 2.57%, 4.83%, and 6.18% tannin levels in 15%, 25%, and 35% concentrations of betel leaf extracts, respectively.

Hagerman [10] reported that tannins could easily bind tightly to proteins to form complex proteins. According to Delak et al. mineralized tooth enamel contains several proteins called amelogenin. Proteins make up approximately 58% of organic material in enamel [11]. This suggests that tannin bonding with proteins in enamel may affect tooth hardness. In enamel, chemical bonds accumulate between tannin and protein, so that when measuring hardness of enamel, that accumulation is covering the surface of the enamel, resulting in decreasing enamel hardness compared with enamel before gel application.

The effect on enamel hardness of tannin contained in betel leaf extract also can be seen in relation to gel. In this study, betel leaf extract was formulated as carboxymethyl cellulose gel. According to Najebb, tannins will form sediment when mixed with gelatin. Decreased enamel hardness after application of the gel also is possible because the enamel tooth surface is covered by a layer of sediment that formed from bonding of gelatin with tannin.

The decrease in enamel hardness also was reinforced by the pH value of the betel leaf extract gel (6.16, 6.16, and 6.43 for 15%, 25%, and 35% concentrations, respectively). From the pH test results, we concluded that the three concentrations of betel leaf extract gel have an acidic pH because of the presence of tannic acid.

The enamel hardness values decreased at concentrations of 15%, 25%, and 35%. The longer the gel exposure, the greater the decrease in hardness value. This was due to longer exposure to tannin acid on the enamel surface, so that there was an increased demineralization reaction. According to Gray, the solubility of enamel caused by acids resulted from the reaction between hydrogen ions and inorganic materials that form enamel. The binding of phosphate ions with H⁺ ions caused the pH of the hydroxyapatite crystals to decrease to 4.5. The pH indicated that erosion occurred and porosity formed in the enamel, thus causing decreased enamel hardness.

An increase in enamel hardness was noted after application of 15% betel leaf extract gel for 6 months. This possibly was due to a reaction with calcium hydroxyapatite, which binds to tannin. In betel leaf extract, 100 mg had a calcium content of 230 mg, which was quite a large amount compared with other substances contained in betel leaf extract. Enamel hardness increased after 6 months of application, so that a reaction between calcium hydroxyapatite and tannin occurred. The bond between the tannin and calcium formed a new layer that showed an increase in values when a hardness test was performed. The delineation was performed on top of the layer, thus resulting in an increased hardness value.

On the basis of one-way ANOVA statistical analysis in comparing the enamel hardness values after application of 15%, 25%, and 35% betel leaf extract gel, there was a significant difference in hardness between before application and after 1, 3, and 6 months. On the basis of a post hoc test, there was a significant difference in the enamel hardness values between betel leaf extract gel concentrations 15% and 25% and 15% and 35% after 1 month; 15% and 35% and 25% and 35% after 3 months; and 15% and 25% and 15% and 35% after 6 months. This possibly was due to the increased tannin levels on the betel leaf extract gel. Tannin content caused lower decreases in enamel.

From this research, an increased concentration of betel leaf extract gel would reduce enamel hardness. The decrease in hardness is associated with the tannin level contained in the betel leaf extracts, thus enhancing the enamel surface porosity. Application of betel leaf extract gel was less effective at increasing hardness of the enamel, either at large or small concentrations and with 6 months of application.

5. Conclusion
Application of 15%, 25%, and 35% betel leaf extract gel resulted in an overall decrease in enamel hardness, with significant differences noted after 1, 3, and 6 months. Although enamel hardness decreased after 1, 3, and 6 months of application, an increase in hardness was noted after 6 months of application of the 15% betel leaf extract.
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