Effect of betel leaf extract gel on color change in the dental enamel

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Abstract: Betel leaf extract gel contains tannin, a chromogenic agent that causes extrinsic discoloration of the tooth enamel. This study aimed to determine the effect of betel leaf extract gel on color changes in the tooth enamel. Eighteen extracted premolar teeth were divided into three groups (n = 6 each) based on the concentration of the betel leaf extract gel: 15%, 25%, and 35%. Color measurements were performed before application, and after 5, 15, and 26 applications (equivalent to 1, 3, and 6 months of application, respectively). The discolorations were calculated using the CIE L*a*b* formula. Statistical analysis revealed significant changes in color on the enamel surface among the 5, 15, and 26 gel applications at all three concentrations. Thus, duration of application of the betel leaf extract gel has a significant effect on color change of tooth enamel.

1. Introduction

The betel plant (Piper betle linn) is one of the most widely-known medicinal plants used by the people of Indonesia. The various types of betel plants include betel Java, betel banda, betel cloves, and black betel [1]. The most commonly used part of this plant is the leaf, which is beneficial for general health and several oral and dental health conditions such as thrush, sore throat, vaginal yeast infection, asthma, halitosis, and dental pain, among others [2]. The antibacterial effects of the essential oil in betel leaf is three times more effective than that of fluorides, because fluorides only serve to inhibit bacterial development and not to destroy them, whereas the essential oil in betel leaf has bactericidal properties and can therefore, maintain the health of the teeth and gums and eliminate bad breath more effectively [3,4].

The essential oil in betel leaves consists of hydroxy kavikol, kavibetol, estargiol, eugenol, methyl eugenol, carvakrol, terpen, sesquiterpen, phenylpropan, and tannin [5,6]. Of these chemical compounds, tannin (polyphenol compound) is known to have an antibacterial effect; [7] however, due to its chromogenic nature it can cause extrinsic discoloration on the tooth surfaces [8].

Owing to the proven beneficial effects of the betel leaf, several products, such as toothpastes and mouthwashes, are currently being produced from these leaves [1]. The study by Sari and Isadiartuti in 2006 demonstrated that at a concentration of 15%, betel leaf extract gel was able to reduce the number of bacterial colonies by up to 57%, whereas at 25% concentration, the microorganisms were completely eliminated [9]. Nevertheless, the use of betel leaves in gel form in humans has not been documented so far.
The present study aimed to evaluate the effect of the gel form of betel leaf extracts, as an alternative to fluoride topical gel, on the color of the dental enamel.

2. Methods
A total of 18 post-extracted human premolars were used in this experimental study. Each dental specimen was inserted into a plastic tube containing a solution of aquades, and was given a specific sequence number.

Dry betel leaf was heated in a water bath at 70°C for 15 min and filtered. The filter water was then heated on waterbath until the powder was extracted. The weight of betel leaf extract was measured to obtain the desired concentration. At the same time, the base material for the gel was prepared by mixing carboxymethyl cellulose (CMC) with the aquades. The measured quantities of betel leaf extract were mixed with the CMC mixture to obtain the betel leaf extract gel (Figure 1).

![Figure 1. Container containing the gel form of the betel leaf extract at concentrations of and 15%, 25%, 35%.](image)

The 18 dental specimens were divided into three treatment groups based on the three concentrations of the betel leaf extract gels prepared; 15%, 25%, and 35%. In each of the groups, 5 times of gel of the first betel leaf gel (equivalent to 1 month of application), 15 next (equivalent to 3 months of application), and 26 subsequent times (equivalent to 6 months of application) were applied.

The VITA EasyShade® Spectrophotometer (VITA Zahnfabrik, Bad Sackingen, Germany) was used to obtain color measurements at each step i.e., prior to gel application, after 5 applications of the first betel leaf extract gel, after 15 applications of the gel, and after 26 applications of the gel. The color was inspected as follows: the tip of the detector was placed perpendicular to the surface to be measured ensuring that it touched the surface to be measured; when the handle was stable, the measuring button was pressed until three “beeps” were heard indicating that the color measurement has been completed. The results of the measurements were displayed on the monitor of the spectrophotometer.

Data analysis was performed using SPSS 17.0. (SPSS 17.0 for Windows; SPSS Inc., Chicago, IL, USA) Repeated analysis of variance (ANOVA) was performed to evaluate significant differences in color measurements based on the duration of gel application, and one-way ANOVA followed by post hoc analysis was used to determine the presence of significant differences between the groups.

3. Results
The values of the color variables of the shade guides including lightness (L*) and the chromaticity coordinates (a*, red-green hue; b*, yellow-blue hue) are shown in Table 1 – 3.
Table 1. Mean L* values of the dental enamel specimens prior to gel application, after 5 applications of the first betel leaf extract gel, after 15 applications of the gel, and after 26 applications of the gel.

| Gel Concentration | L* Before | L* 1 Month | L* 3 Months | L* 6 Months | L* Before | L* 1 Month | L* 3 Months | L* 6 Months | L* Before | L* 1 Month | L* 3 Months | L* 6 Months |
|-------------------|-----------|------------|-------------|-------------|-----------|------------|-------------|-------------|-----------|------------|-------------|-------------|
| 15%               | 78.850    | 84.633     | 5.783       | 82.867      | 4.017     | 81.550     | 2.700       |             |           |            |             |             |
| 25%               | 78.350    | 80.883     | 2.533       | 78.133      | -0.217    | 77.467     | -0.883      |             |           |            |             |             |

Table 2. Mean a* values of the dental enamel specimens prior to gel application, after 5 applications of the first betel leaf extract gel, after 15 applications of the gel, and after 26 applications of the gel.

| Gel Concentration | a* Before | a* 1 Month | a* 3 Months | a* 6 Months | a* Before | a* 1 Month | a* 3 Months | a* 6 Months | a* Before | a* 1 Month | a* 3 Months | a* 6 Months |
|-------------------|-----------|------------|-------------|-------------|-----------|------------|-------------|-------------|-----------|------------|-------------|-------------|
| 15%               | -0.067    | 5.233      | 5.300       | 6.117       | 6.133     | 6.783      | 6.850       |             |           |            |             |             |
| 25%               | -0.400    | 2.200      | 2.600       | 6.983       | 7.383     | 8.167      | 8.567       |             |           |            |             |             |
| 35%               | 0.167     | 2.283      | 2.117       | 6.767       | 6.600     | 7.017      | 6.850       |             |           |            |             |             |

Table 3. Mean b* values of the dental enamel specimens prior to gel application, after 5 applications of the first betel leaf extract gel, after 15 applications of the gel, and after 26 applications of the gel.

| Gel Concentration | b* Before | b* 1 Month | b* 3 Months | b* 6 Months | b* Before | b* 1 Month | b* 3 Months | b* 6 Months | b* Before | b* 1 Month | b* 3 Months | b* 6 Months |
|-------------------|-----------|------------|-------------|-------------|-----------|------------|-------------|-------------|-----------|------------|-------------|-------------|
| 15%               | 26.533    | 39.733     | 13.200      | 39.850      | 13.317    | 41.367     | 14.833      |             |           |            |             |             |
| 25%               | 26.967    | 30.317     | 3.350       | 39.667      | 12.700    | 42.750     | 15.783      |             |           |            |             |             |
| 35%               | 26.017    | 30.950     | 4.933       | 40.167      | 14.150    | 40.667     | 14.650      |             |           |            |             |             |

Color change (E*) values are derived from the mean values of L*, a*, and b*. Table 4 illustrates the increasing trend in E* values as the duration of gel application increased. Increase in E* values was observed during the different steps of gel application (5, 15, and 26 applications) at concentrations of 15%, 25%, and 35%.

Table 4. Mean value of E* on dental enamel specimens after 5, 15, and 26 gel applications.

| Gel Concentration | E* Value 1 Month | E* Value 3 Month | E* Value 6 Month |
|-------------------|------------------|------------------|------------------|
| Betel Leaf 15%    | 15.879           | 16.073           | 17.586           |
| Betel Leaf 25%    | 8.786            | 15.708           | 18.800           |
| Betel Leaf 35%    | 7.630            | 16.039           | 16.995           |

As shown in Table 5, one-way ANOVA test followed by a post hoc analysis revealed no significant difference in E* values after 15 and 26 applications (equivalent to 3 and 6 months of gel application) at all three concentrations. Alternatively, significant changes (p < 0.05) in mean E* values were noted between gel concentrations 15% and 35% after 5 applications (equivalent to 1 month of gel application).
Table 5. Differences in mean E* values between among the three application stages of the betel leaf extract gel at concentrations of 15%, 25% and 35%.

| Concentration | 1 Month | 3 Months | 6 Months |
|---------------|---------|----------|----------|
|               | P       | Explanation | P       | Explanation | P       | Explanation |
| 15%           | 0.056   | No Significant | 0.907   | No Significant | 0.641   | No Significant |
| 15%           | 0.030*  | Significant | 0.990   | No Significant | 0.820   | No Significant |
| 25%           | 0.741   | No Significant | 0.898   | No Significant | 0.489   | No Significant |

Statistically significant difference at p < 0.05

Repeated ANOVA test was used to evaluate the presence of significant differences between the three groups based on the duration of gel application. No significant differences in E* values were noted between the three groups (Table 6). Furthermore, no significant differences in mean E* values were found between the 15 and 26 applications times (equivalent durations 3 and 6 months, respectively) at 25% and 35% gel concentrations, and between the 5 and 15 gel application times (equivalent durations 1 and 3 months, respectively) at 25% concentration.

Table 6. Changes in E* values based on the duration of gel application.

| Equivalent Duration | Concentration 15% | Concentration 25% | Concentration 35% |
|---------------------|-------------------|-------------------|-------------------|
|                     | P                 | Explanation       | P                 | Explanation       | P                 | Explanation       |
| 1 Month             | 0.874             | No Significant    | 0.069             | No Significant    | 0.006*            | Significant       |
| 6 Months            | 0.181             | No Significant    | 0.019*            | Significant       | 0.014*            | Significant       |
| 3 Months            | 0.874             | No Significant    | 0.069             | No Significant    | 0.006*            | Significant       |
| 6 Months            | 0.073             | No Significant    | 0.086             | No Significant    | 0.409             | No Significant    |
| 6 Months            | 0.181             | No Significant    | 0.019*            | Significant       | 0.014*            | Significant       |
| 3 Months            | 0.073             | No Significant    | 0.086             | No Significant    | 0.409             | No Significant    |

Statistically significant difference at p < 0.05

4. Discussion

In the present study, the values of lightness was decreased in most of the treatment groups, which indicated that the color of the enamel surface had changed to a darker shade. On the other hand, the red-green (a*) and yellow-blue (b*) hue values were increased after application of the betel leaf extract gel, which indicated that the colors had turned more red and yellow, respectively. In addition, the CIELAB system, which was used to calculate the overall changes in color, demonstrated an increasing trend in E* values with the increase in the duration of gel application on the dental enamel.
Thus, the longer the gel remained on the tooth surface, the greater the difference in the color of the enamel.

The higher the concentration of betel leaf extract, the higher the tannin content in it. The concentration of tannin in 15%, 25%, and 35% betel leaf extract is 2.57%, 4.83%, and 6.18%, respectively. Significant differences in E* values were observed between the 15% and 35% gel concentrations after 5 applications. No significant differences in color were noted with increase in concentration. This may be due to the fact that the values of the color components before gel application were different; therefore, they can not necessarily be compared between the three groups with different concentrations.

Based on the study by Nordbo H (1977), tannin is a chromogenic material that can cause discoloration of the teeth by binding to the protein contents in saliva [10]. However, in the current study, the specimens did not come into contact with saliva, which might account for the absence of any discoloration.

Furthermore, color change is also known to occur due to the roughness of the tooth enamel, which occurs as a result of exposure to mildly acidic compounds. According to Fujii et al (2011), the roughness of the tooth enamel did not change at a pH of about 6.3 and was considered as neutral [11]. In the present study, the betel leaf extract gel did not have a low degree of acidity. The pH of the 15% and 25% betel leaf extract gels were similar at 6.16, while that of the 35% gel was 6.24. The pH values of the betel leaf extract gels were not lower than the critical pH of the tooth enamel (5.5); therefore, it is possible that the surface roughness of the tooth was not increased following the application of the gel. Based on these findings, we presume that the surface of the enamel may have been rough even before the application of the gel. Deposition of tannins in the betel leaf extract gel on this surface may have resulted in tooth discoloration.

According to the CIELAB theory, a positive a* value indicates a hue of redness. This is thought to occur due to the reaction of tannins with hot water, producing an insoluble red solution called the red body, which might be deposited on to the surfaces of the tooth enamel. A positive b* value indicates a shift toward a yellowish hue. Tannins have been shown to dissolve in water (especially hot water) resulting in a yellowish-brown colored solution [13].

Significant differences in color change (E*) were observed between the 5 and 15 gel applications (equivalent to 1 month and 6 months of application) in the 25% gel concentration group. Similar results were noted between the 5 and 15 gel applications and the 5 and 26 gel applications in the 35% concentration group [12]. Conversely, no significant differences were observed in the 15% concentration group. Based on the CIELAB theory, E* values above 6 indicate a large color change or color difference. In the present study, the longer the application period of the betel leaf extract gel, the higher the value of color change in the dental enamel, which may be attributed to the increase in the deposition of tannins that are in contact with the surface.

5. Conclusion

Significant differences in color change were observed on the dental enamel surface based on the duration of gel application. No significant differences in dental enamel color change were noted among the three different concentrations of betel leaf extract gel (15%, 25%, and 35%).

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