
DISCOLORATION OF TOOTH ENAMEL DUE TO BETEL LEAF EXTRACT (*Piper betle* Linn)

DISKOLORISASI EMAIL GIGI AKIBAT EKSTRAK DAUN SIRIH (*Piper betle* Linn)

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Abstract

The colour of tooth enamel plays an important role in a person's aesthetics. Discoloration of tooth enamel is caused by using mouthwash from the decoction of betel leaves. To date, many Indonesian people are still using the decoction of betel leaves to treat oral diseases and to prevent dental caries. The present study was conducted *in vitro* to determine the effective concentration and exposure duration of betel leaf extract until it can change the colour of tooth enamel. Fresh betel leaves were dried, processed, and macerated so that four concentrations were obtained, namely 50%, 33%, 25%, and 5% while Aquabidest was used as a negative control. Each concentration was examined by soaking two maxillary premolar teeth for each duration, namely 24 hours, 48 hours, and 72 hours. Thirty healthy teeth without growth and development disorders were used in the study. Each concentration of betel leaf extract was found to cause discoloration, and the optimal concentration and immersion duration were 25% and at 48 hours. In conclusion, it is better not to use this ingredient as a mouthwash continuously. Mouthwash from decoction of betel leaves should be used 1 to 2 times a day, each for 2 to 3 minutes.

Keywords: Betel leaf, mouth wash, enamel discoloration

Abstrak

Warna email gigi sangat berpengaruh terhadap estetika seseorang. Perubahan warna email gigi dapat disebabkan oleh penggunaan obat kumur dari rebusan daun sirih. Hingga kini masih banyak masyarakat Indonesia menggunakan rebusan daun sirih untuk mengobati penyakit mulut dan pencegahan karies gigi. Penelitian ini dilakukan secara *in vitro* untuk mengetahui besar konsentrasi efektif serta durasi paparan efektif ekstrak daun sirih sehingga dapat mengubah warna email gigi. Daun sirih segar dikeringkan, diolah, dimaserasi, sehingga didapatkan 4 konsentrasi yaitu 50%, 33%, 25%, dan 5%, serta digunakan akuabides sebagai kontrol negatif. Setiap konsentrasi diteliti dengan merendam masing-masing 2 gigi premolar maksila untuk durasi perendaman 24 jam, 48 jam, 72 jam. Digunakan 30 gigi sehat tanpa cacat tumbuh kembang. Didapatkan setiap konsentrasi ekstrak daun sirih ini menyebabkan perubahan warna, serta konsentrasi optimal dan durasi perendaman adalah dari 25% dan pada 48 jam. Sebagai kesimpulan sebaiknya cara penggunaan bahan ini sebagai obat kumur tidak terus menerus. Disarankan penggunaan obat kumur rebusan daun sirih sebaiknya 1 sampai 2 kali sehari, masing-masing 2 sampai 3 menit lamanya.

Kata kunci: daun sirih, pembersih mulut, perubahan warna email

INTRODUCTION

Teeth enamel covers physiologically open teeth crown into the oral cavity.¹ The surface of tooth enamel influences the aesthetics of a person, especially the facial surface of the maxillary anterior tooth enamel.² Biologically, the enamel is the hardest tooth substance, and it serves to protect the associated teeth crown from various stimuli, especially during mastication.³

Enamel has a thickness of about 2-3 mm. The thickness of enamel varies in different parts of the tooth and between different teeth. Although it is the hardest part of the tooth, the enamel is quite brittle.^{2,4,5} The colour of enamel varies depending on its thickness combined with the degree of mineralization of the tissue below it.¹

The colour of enamel is permanent from yellowish-white to greyish white, whereas the enamel of deciduous teeth is bluish-white^{2,4,5} or looks opaquer. This situation is caused by slightly less mineralized deciduous teeth than the permanent teeth that replace it.¹ In the enamel tissues, discoloration is easy to occur², either because of intrinsic and extrinsic factors or local and systemic factors.⁶

To date, various mouthwash can be found, both chemical and herbal.^{7,8} One of the herbs that are widely used in Asia and frequently studied is betel leaves (*Piper betle*). Indonesian people use a betel leaf decoction as a mouthwash to cope with gingiva inflammation (gingivitis) and bad breath (halitosis) and to prevent dental caries.^{7,9,10} Researchers in Indonesia have made natural mouthwash from betel leaf extract with the same bactericidal effectiveness as a chemical mouthwash.¹¹

Ethanol extract from betel leaves contains many useful phytochemical ingredients. Some of them are essential oils, tannic acid (tannin), chavibetol, methyl eugenol, hydroxychavicol, and others.^{9,12,13} Tannin is also known to be abundant in tea and coffee.⁸ Tannin in tea gives a brown colour. It is known that tea causes discoloration on teeth, and the discoloration occurs due to the content of tannins within the tea.¹⁴ Ashok and Upadhyaya (2012)¹⁵ also wrote that tannins cause discoloration on the teeth so that it interferes with the aesthetics of individuals.

The present study was conducted to determine the effect of the tannin content in betel leaf extract and the duration of its exposure to discoloration in tooth enamel.

MATERIALS AND METHODS

This research was a laboratory experiment with a pre-post control group and single-blind research design. The research was conducted at the Biochemistry Laboratory of the Faculty of Medicine, University of YARSI, Jakarta. Fresh green betel leaves were purchased from sellers of medicinal plants in a traditional market.

The research has been approved by the Research Ethics Committee of the YARSI University Research Institute with number 282/KEP-UY/BIA/XI/2016. Betel leaf extract was used with a concentration of 50%, 33%, 25%, and 5%, while Aquabidest was used as the negative control. The discoloration was observed in 30 new maxillary premolar teeth extracted from patients who needed orthodontic treatment. The teeth were in healthy or intact conditions, and there were no structural abnormalities due to growth and development disorders.

Fresh betel leaves were cleaned, chopped, dried under the sun, and heated at 36°C for 3 hours. Dry betel leaves were weighed to obtain four concentrations, namely 50 grams, 33grams, 25 grams, and 5 grams unit of dry weight. The leaves in each group were blended to obtain a powder form. Each powder was soaked with 100 ml of 96% ethanol liquid for 24 hours in a dark glass tube and then put in the oven at room temperature (25-27°C). This maceration process caused the solution to become concentrated. The betel leaf extracts with a concentration of 50%, 33%, 25%, and 5% in 100 ml of 96% ethanol were obtained.

Two maxillary premolar teeth specimens were put into fifteen 30 cc plastic pots. 6 ml betel leaf extract with different concentrations was poured into each pot, while 6 ml Aquabidest was filled in the last pot as a control. In addition to different concentrations, each pot had a different duration of immersion, such as 24 hours, 48 hours, and 72 hours. All research pots were kept at a temperature of about 36°C in an oven to match the temperature of the oral cavity.¹⁶

From each tooth tested, the colour was measured before and after the treatment by using Vita easy shade guide classic, each for 5 seconds. The colour was determined by two different trained observers. Data were analyzed by the SPSS program using one-way ANOVA test to determine the difference in the concentration of each time group. A post hoc LSD (Least Significant Difference) test was carried out to determine the differences in the results of each concentration group at the same time. The confidence level was 95%.

RESULTS

Normality test was carried out regarding the duration of and concentration of the betel leaf extract solution and Aquabidest by using the Shapiro-Wilk test. The p-values obtained for the duration of immersion were 0.393, 0.3, and 0.78, whereas, the p-values obtained for the concentration of solution were 0.610, 0.804, 0.313, and 0.540, and Aquabidest had a p-value of 0.042. All p-values were greater than 0.05 (except Aquabidest), which means that the data distribution of the two independent variables was normal. Thus, data processing was done by parametric tests.

Data about the colour state of all teeth before and after the treatment were compared and analyzed by paired T-test (Table 1). Data were obtained as observation results by two trained operators. The results showed that there were significant differences in the enamel colour of all teeth tested before treatment and after the treatment.

Table 1. Colour state of tooth enamel before and after the treatment

Colour state	Mean difference	SD	N	p
Before	6.8500	1.6976	30	0.0000
After	10.3333	2.0982		

Description: $\alpha = 0.05$

Similarly, one-way Anova test, which was carried out to see discoloration between concentrations in each group of immersion duration, found a significant difference (Table 2)

Table 2. The average discoloration between each concentration at each duration of immersion

Duration of immersion (hours) according to concentration and control	Mean after treatment	p
24	5.123	0.011
48	9.475	0.035
72	12.350	0.026

Description: $\alpha = 0.05$

A post hoc LSD test was used to test the colour differences in each duration of immersion among the concentration groups, with a confidence level of 5% (Table 3). This table shows the average difference in colour according to the duration of immersion between the two concentration groups and control group.

From all durations of immersion, the largest and most significant colour difference was found in the 25% concentration solution. In terms of the duration of immersion, the 24 hours of immersion showed the largest mean difference with the control group (3.750), whereas the largest mean difference in the 72 hours of immersion was found in the concentration of 5% (6.000) and control (5.500).

Table 3. The difference in colour between concentration and control of each duration of immersion

Duration of immersion (hours)	Concentration group (%)	Mean difference	p
24	50	33	0.000
	50	25	0.129
	50	5	0.022
	50	K	0.015
	33	25	0.129
	33	5	0.022

33	K	1.250	0.015
	5		0.004
	K	2.250	0.003
	K		0.732
		2.500	
		3.500	
48		3.750	
			0.250
	50	33	-0.750
	50	25	-1.500
	50	5	1.500
	50	K	4.000
72	33	25	-0.750
	33	5	2.250
	33	K	4.750
	25	5	3.000
	25	K	5.500
	5	K	2.500
72	50	33	3.250
	50	25	2.750
	50	5	3.250
	50	K	2.750
	33	25	-5.000
	33	5	1.000
72	33	K	0.500
	25	5	6.000
	25	K	5.500
	5	K	-0.500
			0.717
			0.717

Description: $\alpha = 0.05$

K = control

The viability of absorbance was measured, or the absorption capacity of the betel leaf extract solution used to soak the teeth was compared to the betel leaf extract solution which was not used to soak the teeth. The results showed that the highest absorbance of the main ingredients of betel leaf extract was in the 48 hours of immersion (Table 4). Measurements were made using a UV-VIS spectrophotometer with λ 608 nm.

Table 4. The average absorbance of betel leaf extract in all four concentrations according to the duration of immersion

Duration of immersion (hours)	Average absorbance
24	12.25
48	18.75
72	10.25

DISCUSSION

All 15 plastic pots, each containing two elements of premolar teeth and the soaking material of betel leaf extract with different concentrations or Aquabidest liquid as a control (four for each duration of immersion) were stored in an incubator at 36⁰ C. This temperature was used because it was suitable with the temperature of the oral cavity, 36-37⁰ C.¹⁶ This was done as an attempt to control confounding variables that might interfere with the process and results of the study.

The Vita easy shade guide, commonly used in clinic/dental clinic or denture manufacturing laboratory, was used to determine the enamel colour and its changes. The Shade guide has 16 different colours. The colour was measured visually within five seconds for determination of each colour. To reduce the subjectivity of this method, the colour was determined in a single-blind manner by two different trained observers who underwent the same perception before the study was conducted.

Betel leaves are known to have various pharmacological benefits, such as anti-fungal, anti-inflammatory, immune modulator, and anti-microbial drugs.^{9,13,17} *Streptococcus mutans* is one of the bacteria that can be influenced by betel leaf extract with a bactericidal role. As known, these bacteria play a role in causing halitosis and dental caries.^{9,10} Many types of mouthwash from this herb are used by the community because it has been shown to reduce dental plaque formation.^{7,8}

Syamsuhidayat and Hutapea (1991)¹⁸ wrote that betel leaves are widely used for the treatment of tooth and mouth pain, mouth ulcers, oral abscesses, and tooth extraction wounds. It has also been widely used to treat coughing and hoarseness, bloody nose, itching, dizziness, stomach disorders, haemorrhoids, vaginal discharge, palpitations, trachoma, and eye drops. Betel leaf extract has many benefits. However, it causes discoloration in tooth enamel. Herdiyati et al. (2015)¹¹ and Karlina (2016)¹⁷ stated that tannins in betel leaves play a role in the discoloration process of tooth surfaces.

Tannin is a water-soluble polyphenol compound, commonly found in plant foods.¹⁹ This alkaline material cannot crystallize, but it can oxidize oxygen in the water to form colloidal solutions that provide a sour and spongy taste. Tannin can precipitate proteins from other compounds and form complexes with these proteins. These complex compounds cannot be broken down by proteolytic enzymes.²⁰ This material has astringent and antiseptic action and gives colour to other ingredients. Tannin can be identified by a chromatographic procedure.²¹

The study results indicate that there was discoloration in enamel due to exposure to betel leaf extract from all four concentrations. This can be seen in Table 1 (discoloration before treatment were compared with each stage after treatment of each tooth at each betel leaf extract concentration) with $p = 0.000$. This is also proven in Table 3 (between each of the two concentrations or with the control group) and Table 2 (between concentrations and the control) of each duration of immersion.

As stated earlier that tannins, for example from tea, cause discoloration in the drinkers' teeth.¹⁴ Moreover, the research results by Dinna et al. (2013)²² found that betel leaf extract tannins were the cause of tooth discoloration. They used it in gel form. Ashok and Upadhyaya (2012)¹⁵ also stated that the tannin content in betel leaf extract is the main cause of tooth discoloration. Based on this research, it can also be concluded that the cause was tannin content from the research solution.

Dinna et al. (2013)²² stated that the higher the concentration of betel leaf extract, the higher the level of tannin in it. This finding is based on their research results that the discoloration was more evident in the use of gels from higher concentrations of betel leaf extract. However, as seen in Table 3, in each duration of immersion group, it was found that the concentration of betel leaf extract 25% had the greatest colour difference, especially for the control group. As discoloration was mainly due to tannin content, it means that the tannin level in the concentration of betel leaf extract 25% in this study was the most effective in causing discoloration of tooth enamel. Thus, this means that discoloration is not caused by high levels of tannin in a solution.

Table 4 shows the average absorbance of betel leaf extract solution from the four concentrations according to the duration of immersion, in which the largest was found in the 48-hour group. Then, the average absorbance decreased in the 72-hour group, and this value was smaller than the 24-hour group. This condition indicates that the absorption of colour or tannin at the beginning of the 24 hours of immersion was quite high, then it increased to optimal at 48 hours of immersion.

The tannin content decreased at 72 hours of immersion. This is evident from the average absorbance value (10.25), which was also lower than the initial absorbance or at 24 hours of immersion (12.25). It is estimated that if the research period is extended, the average absorbance value will be much lower, or the discoloration will be minimal. It can be concluded that the continuous use of betel leaf extract as a mouthwash causes discoloration of tooth enamel, regardless of the concentration.

The effective concentration of discoloration with continuous exposure was 25% with the optimal time at 48 hours. Although this material is useful as an oral bactericide, astringent, and antiseptic, it is preferable that users boil and rinse their ingredients by checking the times of use to minimize the negative impact of enamel discoloration, such as intermittent

use which means 1 to 2 times a day after breakfast and before sleep at night, each for 2 to 3 minutes according to the recommendations of the Basic Health Research in 2013.²³ Further research is also needed to ascertain the relationship between the concentration of betel leaf extract with the material absorbance, and the intermittent use conditions.

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