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BLEACHING EFFECTS AFTER THE REPEATED APPLICATION OF 2.5% *CITRUS RETICULATA BLANCO* PEEL EXTRACT GEL

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ABSTRACT : Tooth with discoloration can undergo bleaching to restore their natural white color. Previous research revealed that the repetition of hydrogen peroxide application enhances the effectiveness of teeth whitening, but the use of this substance may lead to side effects. A 2.5% tangerine peel extract gel is known to have the potential as a teeth-whitening material, but the impact of repeated application of the gel on teeth whitening effectiveness is yet to be determined. Objective: To determine the effect of repeated application of 2.5% tangerine peel extract gel on the degree of teeth whitening. Material and method: Experimental research involved 24 premolar teeth with a C4 shade, divided into four groups: Treatment Groups A and B were applied with 2.5% tangerine peel extract gel, while positive control Groups C and D were treated with 40% hydrogen peroxide. The application was repeated twice in Groups B and D. Tooth color was measured before and after the application of the bleaching material using colorimeter, and the resulting ΔL*, Δa*, Δb*, and ΔEab* values underwent statistical analysis using One-Way ANOVA and Post Hoc LSD tests. Results: No significant difference was observed in Δa* and Δb* values among all groups. The group subjected to repeated bleaching applications exhibited significantly higher brightness (ΔL*) and total color change values (ΔEab*) compared to the other groups. Conclusion: The repeated application of 2.5% tangerine peel extract gel enhance the degree of teeth whitening. However, it has not reached the same degree achieved with the application of 40% hydrogen peroxide.

KEYWORDS : *tangerine peel extract, teeth whitening degree, tooth bleaching.*

INTRODUCTION

Color plays a crucial role as a factor influencing the aesthetic outcomes of dental care. Bright natural tooth color reflects a healthy oral cavity and can have an impact on psychosocial aspects.[1] However, teeth can undergo color changes referred to as tooth discoloration, which can be classified into intrinsic and extrinsic discoloration. Intrinsic discoloration is caused by changes within the tooth structure. The alterations in the tooth structure result from factors such as aging,

chromogenic substances from specific food consumption, micro-cracks in the enamel, tetracycline medication, systemic diseases like porphyria, genetic disorders, excessive fluoride consumption, tooth trauma, and cavities.[2] Meanwhile, extrinsic discoloration constitutes a color alteration due to the presence of stains adhering to the surface of tooth enamel. It can result from substances like tannins found in beverages such as tea and coffee, from tobacco smoking, and the accumulation of plaque and calculus, which can impart a dark brown coloration to the teeth.[3]

Teeth with discoloration can be treated through the dental bleaching process to eliminate stains on the teeth using a whitening agent, which operates through an oxidative chemical reaction. This agent reacts by breaking the organic bonds of stains on the tooth surface, resulting in a whiter appearance of the teeth. The bleaching procedure applied to vital or non-vital teeth with extrinsic discoloration on the enamel surface is called extra-coronal bleaching. There are two types: in-office bleaching and at-home bleaching, which utilize hydrogen peroxide or carbamide peroxide bleaching agents.[4]

The acidity level (pH) of bleaching agents is one of the factors influencing the success of the bleaching procedure. Hydrogen peroxide has an acidic pH of around 4-5, exceeding the critical pH of enamel, which is approximately 5.5. The acidic nature and the presence of radical compounds dictate that the concentration of bleaching agents is generally adjusted based on the duration of application.[5] High concentrations of carbamide peroxide (30%-45%) are used in in-office bleaching procedures with application duration ranging from 30 to 60 minutes.[6]

In the case of in-office bleaching procedures using high concentrations of hydrogen peroxide, specifically 40%, it has been reported that the application on teeth can be as short as 20 minutes. According to the manufacturer's instructions, the application of hydrogen peroxide bleaching agent with a concentration of 40% for a duration of 20 minutes can be repeated up to three consecutive times.[7] However, the use of hydrogen peroxide bleaching agents has been reported to cause several side effects, including increased tooth hypersensitivity, irritation of the gingiva, induction of morphological changes in enamel such as increased surface roughness, increased groove depth, and alterations in micro-hardness of enamel, thereby facilitating plaque formation.[8]

Natural ingredients are increasingly being developed as alternatives to chemical bleaching agents, one of which is the tangerine (*Citrus reticulata blanco*). This is due to the presence of various organic acids in its peel, including citric acid and gallic acid.[9]

The *C. reticulata blanco* cultivated in Indonesia is a type of mandarin orange belonging to the Rutaceae family. The cultivation of this orange is located in Batu City, East Java Province. This is what makes this type of orange in Bahasa Indonesia known as "jeruk keprok Batu". The Citrus and Subtropical Fruit Research Institute (Balai Penelitian Tanaman Jeruk dan Buah Subtropika or Balitjestro) has registered this orange seedlings and has been officially recognized by the Ministry of Agriculture of the Republic of Indonesia through Decree No.

307/Kpts/SR.120/4/2006 as a superior national variety under the name "Jeruk Keprok Batu 55".[10]

Citric acid can penetrate the tooth surface, releasing stains that cause tooth discoloration. The extracted peel of *C. reticulata blanco* has been reported to contain approximately 136.6 ± 0.1e µg/g of gallic acid through the maceration extraction method.[11] Gallic acid is known to be a phenolic acid that can react to form quinone compounds, semiquinones, and hydrogen peroxide, potentially acting as a free radical oxidizer for breaking the bonds of stains on discolored teeth.[12,13]

Pratiwi et al. indicate that teeth whitening is significantly achieved by immersing teeth in *Citrus reticulata blanco* peel extract with concentrations of 2.5%, 5%, and 10%. The lowest degree of teeth whitening is obtained from the smallest concentration of peel extract, which is 2.5%, compared to the teeth whitening results from a concentration of 5% during a 30-minute application.[9] Additionally, the teeth whitening results from the application of bleaching agents are known to be enhanced with repeated applications. Some studies suggest that, with the application of a 40% hydrogen peroxide bleaching agent, the best teeth whitening results and the lowest mineral content are achieved through a 20-minute application, with the process being repeated up to two times.[14]

Repeated application of *C. reticulata blanco* peel extract is expected to increase the degree of teeth whitening. The duration of the application of *C. reticulata blanco* peel extract needs to be adjusted because, according to previous research, the citric acid it contains can cause enamel demineralization with exposure durations of 30 consecutive minutes and in highly acidic pH (3.2).[15] The selected duration for the application of *C. reticulata blanco* peel extract in this study is 20 minutes, and formulated into a gel preparation to facilitate application on the tooth surface. The selection of the extract concentration in this study is 2.5%, which is the lowest concentration from previous research to minimize the potential damage to tooth structure if the application of the bleaching agent is repeated. So far, it is not known how much teeth whitening is achieved from the repeated application of calamondin peel extract gel. Therefore, further research is needed to evaluate the teeth whitening results from the application of 2.5% *C. reticulata blanco* peel extract gel with a 20-minute application duration, repeated twice.

METHOD

This study is an experimental research with a pre-test and post-test group design, conducted in the Basic Chemistry Laboratory of the Faculty of Medicine and the Chemical Technology and Microbiology Laboratory of the Agricultural Technology Faculty at the University of Sriwijaya. The subjects of this research are first and/or second premolar maxillary and/or mandibular without caries. The sample size was calculated using Lemmeshow formula. Based on the formula, the minimum number of samples in one research group is 6, making a total of 24 samples divided into four groups: Group A and B were treated using 2.5% *C. reticulata blanco* peel extract gel once and twice, respectively. Group C and D were positive control group treated with 40% hydrogen peroxide once and twice, respectively. The duration for each application was 20 minutes.

Specimen preparation

The discoloration procedure on tooth specimens was conducted by immersing 24 premolar teeth in an arabica coffee solution. Before immersion, the tooth specimens were coated with transparent nail polish three times on the entire root surface up to the Cemento Enamel Junction (CEJ). The arabica coffee solution was prepared by mixing 10 grams of coffee powder with 200 mL of 100°C distilled water. Immersion was carried out at a temperature of 37°C. Every 24 hours, the solution was replaced, and the tooth immersion was repeated until the teeth reached a color equivalent to shade C4.

A separating disk bur was used to create a separation at the cemento-enamel junction (CEJ). Polyester resin disc molds were crafted to have a thickness and diameter of 2 cm. These molds were coated with a separating medium. Premolar teeth were implanted with the buccal part facing upward. The entire acrylic disc was polished using a cotton wheel bur coated with pumice paste. Next, the color values of the teeth, represented by the initial L*, a*, and b* values, were measured using a colorimeter and recorded.

Preparation of *C. reticulata blanco* peel extract gel

The orange peel is cut into small pieces, dried in an oven at 50ºC, and then ground into powder using a blender. Next, 2 grams of the orange peel powder is mixed with a solvent, which consists of 25 ml of methanol and 75 ml of distilled water, in a glass beaker. The peel extract solution is heated at 50ºC until it becomes dry extract. The bleaching gel was made by mixing the dry peel extract with a gelling agent, 2% sodium carboxymethyl cellulose (Na CMC), totaling 100 grams, resulting in a concentration of 2.5% *C. reticulata blanco* peel extract gel.

Bleaching agent application

Before applying the bleaching agent, all specimens were stored in artificial saliva medium at a temperature of 37ºC for 2x24 hours. Specimens in groups A and B were treated with 2.5% C. reticulata Blanco peel extract gel, while groups C and D were treated with 40% hydrogen peroxide bleaching agent (Halodent Dental Opalescence PF Boost Bleaching Ultradent).

The bleaching agent is applied to the entire surface of the tooth specimen with a thickness of approximately ±2 mm for 20 minutes per application. In the groups with repeated applications, the bleaching agent from the first application is cleaned using gauze and running water until it is completely clean, and directly reapplied using the same procedure. Subsequently, tooth color measurements are taken.

Tooth color measurement

The degree of teeth whitening was measured using the CHNSpec WR-10 colorimeter with a black-colored paper background on a flat surface. Measurements were taken before and after the application of the bleaching agent on the buccal surface of premolar teeth, based on the CIELAB parameters, namely L*, a*, and b. The degree of teeth whitening from the colorimeter measurements was calculated using Microsoft Excel with the installed CIELAB scale, which includes values for

ΔL*, Δa*, Δb*, and ΔEab*. The values of the degree of teeth whitening were recorded for further data analysis.

RESULTS

The results of the bleaching agent application are presented in Table 1 below, showing the values for the color change of the teeth. The data for ΔL*, Δa*, Δb*, and ΔEab* in each group underwent the Shapiro-Wilk normality test and Levene's homogeneity test, with the results indicating normal distribution ($p > 0.05$) and homogeneity ($p > 0.05$).

Table 1. The values for color change post-application of the bleaching agent.

	Group A	Group B	Group C	Group D
Mean values				
ΔL*	8,42	11,70	14,79	18,27
Δa*	-3,00	-3,44	-3,80	-3,89
Δb*	-6,77	-8,78	-8,71	-10,07
ΔEab*	11,54	15,14	17,74	21,55

Subsequently, the data underwent One-Way ANOVA test to determine if there was a significant difference in the averages among groups.

Table 2. The results of the One-Way ANOVA test for the values of ΔL*, Δa*, Δb*, and ΔEab*.

Value		N	Significance
ΔL*	Between groups	24	**0,000***
Δa*		24	0,671
Δb*		24	0,237
ΔEab*		24	**0,000***

Based on the results of the One-Way ANOVA test in Table 2, it is evident that the differences in the average values of Δa* and Δb* among treatment groups do not show significant differences. Significant differences in average values are observed in the brightness change of tooth color (ΔL*) and the total color change (ΔEab*) among treatment groups. The statistical analysis is further continued with a post hoc test on the values of ΔL* and ΔEab*, the results showed in Table 3.

Table 3. The results of Post Hoc LSD of ΔL* values

Groups	**A**	**B**	**C**	**D**
A	-	**0,021***	**0,000***	**0,000***
B		-	**0,029***	**0,000***
C			-	**0,015***
D				-

Based on the follow-up Post hoc LSD test presented in Table 3, significant differences are observed in the average brightness change of tooth color (ΔL*) among each research group. The total color change of teeth (ΔEab*) presented in Table 4 indicates significant average differences among each research group except

between groups B and C.

Table 4. The results of Post Hoc LSD of ΔEab* values

Groups	A	B	C	D
A	-	**0,012***	**0,000***	**0,000***
B		-	0,059	**0,000***
CD			-	**0,008***
				-

DISCUSSIONS

In this current study, colorimeter is used in measuring the increase in the degree of teeth whitening because it is more accurate compared to the shade guide, which has shortcomings such as being subjective and prone to the possibility of metamerism.

The results of this study indicate that the application of 2.5% *C. reticulata blanco* peel extract gel in groups A and B, as well as 40% hydrogen peroxide in groups C and D, resulted in an increase in the brightness of tooth color or L* values, as indicated by positive ΔL* values. It is evident that the groups with repeated applications of the bleaching agent (B and D) have significantly higher changes in tooth color brightness (ΔL*) compared to the groups with a single application of the bleaching agent (A and C).

The a* and b* values on the color scale respectively represent the red-green and yellow-blue axes on the teeth. All research groups have negative Δa* values, indicating a decrease in the degree of red color on the teeth. The negative Δb* values in all groups also indicate that the teeth's color axis is shifting towards the -b* value or bluish, visually reducing the degree of yellowness on the teeth.[14] The decrease in the values of a* and b* along with the increase in the value of L* on the teeth can be interpreted as an increase in the degree of whitening when observed visually.[17,18]

Studies assessing the effectiveness of teeth bleaching commonly use the ΔEab* value as a measure of total color change. This value represents the degree of color change in an object. In line with Ozdemiz et al.'s, our study found that groups which had repeated bleaching applications, showed significantly higher ΔEab* values compared to groups with a single application.[7] Group D, receiving repeated applications of 40% hydrogen peroxide, had the highest total color change. However, the difference in ΔEab* values between groups B and C was not statistically significant. This suggests that the total color change from repeated applications of 2.5% *C. reticulata blanco* peel extract gel is not significantly different from the color change resulting from a single application of 40% hydrogen peroxide.

The whitening ability of *C. reticulata blanco* extract in this study can be attributed to its citric acid content. Radhakrishnan et al. stated that citric acid in strawberries can penetrate tooth enamel, removing discoloration-causing stains and resulting in whiter teeth.[19] Furthermore, *C. reticulata blanco* extract contains gallic

acid, which can be oxidized into hydrogen peroxide, playing a role in the teeth-whitening process.[20-21]

The ideal bleaching application is one that achieves the most effective teeth whitening with minimum changes in mineral structure on the enamel. The repeated application of 2.5% *C. reticulata blanco* peel extract gel with a pH of 6.4 in this study has not been definitively established for its effects on tooth enamel structure. The repetition of bleaching applications itself may have impact on the tooth surface. Further research is therefore necessary to assess the optimal teeth whitening results from repeated applications and the pH of 2.5% *C. reticulata blanco* peel extract gel, as well as its effects on tooth enamel structure.

CONCLUSION

Based on the results, the following conclusions can be drawn:

a. The application of 2.5% *C. reticulata blanco* peel extract gel twice can enhance the brightness values (ΔL*) and total color change of teeth (ΔEab*) to a greater extent compared to a single application.
b. The application of 40% hydrogen peroxide demonstrates higher brightness values (ΔL*) and total color change of teeth (ΔEab*) compared to the application of 2.5% calamondin peel extract gel.

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