

Tooth Extrinsic Stain Remover By Averrhoa Bilimbi L Extract Solution

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ABSTRACT

Background : Dental stains are pigmented deposits that can be a predisposing factor for periodontal disease and caused aesthetic problems. Chromogens from foods and beverages such as coffee can adhere to the tooth surface through pellicle and forming deposits called tooth extrinsic stains. Averrhoa bilimbi L contains oxalic acid which has potential ability to remove it through oxidation mechanisms. The aim of this study was to investigate the effectiveness of Averrhoa bilimbi L extract solution on extrinsic tooth stain removal.

Method : Tooth extrinsic stain models obtained by soaking the teeth in robusta coffee solution. Twenty-four post extraction premolar teeth with extrinsic stains were divided into 4 groups consisting of control group (immersion in artificial saliva), T1 group (immersion in Averrhoa bilimbi L extract solution 50%), T2 group (immersion in Averrhoa bilimbi L extract solution 75%) and T3 group (immersion in Averrhoa bilimbi L extract solution 100%) for 28 hours. Adobe Photoshop CC 2018 used to evaluate the difference color intensity (ΔE) in CIE LAB system before and after the treatment.

Result : Anova test showed there's a significant difference within groups ($p < 0.05$). Post Hoc Post test showed there are significant difference of difference color intensity (ΔE) between each group ($p < 0.05$), with the highest average of it on T3 group then followed by T2 group, T1 group and control. Pearson correlation analysis showed there is a positive correlation between concentration and color intensity.

Conclusion : Averrhoa bilimbi L. extract solution proven effective as extrinsic stain removal in vitro.

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INTRODUCTION

Increased appearance satisfaction has an impact on improving the quality of life and condition psychologically, this is closely related to the aesthetics of the teeth as well. Factors affecting dental aesthetics are: tooth color, tooth position, shape and number of teeth, also a person's dissatisfaction with the appearance of teeth, in part mostly caused by discoloration of the teeth¹. Dental stains were pigmented deposits found on the surface of the teeth, these conditions are often associated with health problems in the oral cavity and become aesthetic problems in daily life. Dental stains can be divided into two type based on the cause, namely intrinsic stains (originating from the inside) and extrinsic stains (originating from the outside). Extrinsic stain was found on the surface of the tooth and has relation with pellicle staining or chemical interactions of a substance. There are several causes of dental stains, including: smoking habits, consumption of colored food and drink, poor oral hygiene or the use of chemical mouthwash in the long term such as chlorhexidine gargle. Coffee is one of the colored drinks that able caused tooth extrinsic stain. It caused by the presence of chromogenic material in coffee that reacts with plaque or pellicle on the surface of the teeth²³⁴.

The incidence of periodontal disease in both developed and developing countries is still quite high and affect about 20-50 % of global population, the higher prevalence of periodontal disease in adolescents, adults and the elderly makes it as a public health problem⁵. Dental stain is one of the predisposing factors of periodontal disease, with dental plaque as the main etiology. The presence of it causes roughness on the surface of the teeth which results in the authenticity of dental plaque in that area, it can trigger inflammation in the periodontal tissue. Removal of dental deposits such as calculus, dental plaque or dental stain usually was done by clinicians with a scaling-root planing procedure in daily practice, But those main therapy have some disadvantages as follow : the cost is quite expensive, it has a risk of causing discomfort to the patient, the procedure can cause bleeding in the gingiva during treatment or sometimes it unable to get optimal result because there are parts that cannot be reached by tools. This condition needed adjunctive therapy such as systemic or oral antimicrobials to improve the outcome of main therapy⁶. It also needs to be considered an alternative treatment which can prevent periodontal disease and overcome that problems, such as self-prevention efforts like toothbrushes or the use of mouthwash.

Currently the use of natural ingredients is quite popular in the community because it is considered cheaper, safer and minimal side effects. Indonesia has abundant natural wealth, but its utilization has not been widely used optimally. *Averrhoa bilimbi* L is one of the natural plants which derive from Southeast Asia including Indonesia. *Averrhoa bilimbi* L is easy to find, relatively inexpensive, and can bear fruit all year round. *Averrhoa bilimbi* L contains oxalic acid and peroxide compounds that are thought to be able to restore the white color of teeth. Iskandar et al stated that oxalic acid contained in spinach leaf extract and milk solution can reduce tooth staining's are caused by coffee⁷. Fauziah et al stated that *Averrhoa bilimbi* L extract gel concentration 100% effective as an alternative to natural bleaching ingredients⁸. Rahaju et al state that 50% *Averrhoa bilimbi* L extract gel affects in increasing the degree of brightness of the teeth in vivo in rabbits' study⁹. So far there has been no further research on *Averrhoa bilimbi* L extract solution in some concentration and its effect on tooth extrinsic stain. This study was conducted to determine the effectiveness of *Averrhoa bilimbi* L against the removal of extrinsic stains of teeth.

METHODS RESEARCH

Research Design

This study was true experimental design research and randomized pre-test and post-test Control Group Design. This research has been evaluated and approved by Health Research Ethics Committee, State Polytechnic of Health Malang (certificate Reg.No.:483/KEPK-POLKESMA/2019)

Preparation of Averrhoa bilimbi L extract solution

Averrhoa bilimbi L extract was made using maceration method with 70% ethanol solvent. Previously Averrhoa bilimbi L fruit is thoroughly washed and aerated, later it thinly sliced and soaked in 70% ethanol in a ratio of 1: 2 for 3 x 24 hours in a closed container, furthermore it was filtered¹⁰. The filtrate of Averrhoa bilimbi L extract was concentrated with a rotary evaporator, then it was diluted using aquadest to obtain a cooling concentration.

Sample preparation

The samples in this study were 24 post-extraction premolars with the following inclusion criteria: there is not any dental caries, restoration, dental calculus or cracked/fractured tooth. Each sample was soaked in saline solution, and it was cleaned using a brush and pumice. After the entire surface of the tooth was clean, a transparent nail polish was applied to the root and crown except the buccal side. Furthermore, each sample soaking in the artificial saliva for 2 minutes, it was considered to facilitating the formation of pellicle as a dye chromogen attachment medium in the formation of extrinsic stain. The next step was numbering on each sample then it was placed in the container.

Dental extrinsic stain models is done by soaking teeth into robusta coffee solution for 14 days at room temperature, coffee solution replacement was done every 24 hours^{8 11}. After the extrinsic stain was formed, it was characterized by a brownish layer on the teeth. The treatment was carried out by dividing the samples into 4 groups with a total of 6 samples for each group. The division of such groups is the control group (immersion in artificial saliva), T1 group (immersion in a solution of *Averrhoa bilimbi* L extract at concentration 50%), T2 group (immersion in solution of *Averrhoa bilimbi* L extract at concentration 75%) and T3 group (immersion in solution of *Averrhoa bilimbi* L extract concentration at 100%). Samples immersion in the control and treatment groups were carried out at room temperature for 28 hours in plastic container.

Calculation of difference color intensity (ΔE)

Each sample was taken a picture before and after the treatment. The photo shoot was done in a mini studio with LED lights at the top and white background. Samples were photographed using a Samsung Galaxy S10 smartphone with manual setting (ISO 50, f/2.4, focal length 4.32 mm, shutter speed 8 s) at a fixed distance of 20 cm. The images were analyzed using Adobe Photoshop CC2018 software to determine the difference color intensity (ΔE) of samples. The values of L, a, and b were obtained for each sample before and after treatment. Furthermore, the measurement of the difference color intensity (ΔE) values were carried out with the calculation formula of CIELAB with CIEDE2000 Method to avoid refraction¹². According to Isa and Pradana, the results of color measurements in CIELAB images have similarities with human vision perception¹³.

$$\Delta E^* = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Information:

ΔE^* = Total color change

ΔL^* = difference between light and dark (+ = lighter, - = dark)

Δa^* = difference of red and green (+ = red, - = green)

Δb^* = difference between yellow and blue (+ = more yellow, - = blue).

Data analysis

Data analysis was performed as follow; normality test was done using Shapiro-Wilks test and homogeneity test was done using Levene test, Furthermore One-way Anova and Post hoc multiple comparison tests were performed to determine the significance of differences between each group.

RESULT

The average value of the difference color intensity (ΔE) of teeth between before and after treatment along with the standard deviation can be seen in table 1 and figure 1. The 100% *Averrhoa bilimbi* L.

Normality and homogeneity test showed that the data are normally distributed and homogeneous. One Way Anova test showed the value of $p = 0.00$ ($p < 0.05$), it means that there is a significant difference of mean values in difference color intensity (ΔE). Post Hoc Multiple Comparison test showed a value of $p < 0.05$ in each group (table 2). The condition of the teeth before and after treatment can be seen in figure 2 clinically.

Table 1. Average numbers of difference color intensity (ΔE) $\pm$ SD before and after treatment

Groups	Number of samples	difference color intensity (ΔE) $\pm$ SD	P
Control	6	2.9 $\pm$ 0.48	0.000*
<i>Averrhoa bilimbi</i> L 50% (T1)	6	6.7 $\pm$ 0.83	
<i>Averrhoa bilimbi</i> L 75% (T2)	6	8.3 $\pm$ 0.60	
<i>Averrhoa bilimbi</i> L 100% (T3)	6	12 $\pm$ 1.4	

*Significant difference (One-way Anova : $p < 0.05$), SD: Standard Deviation

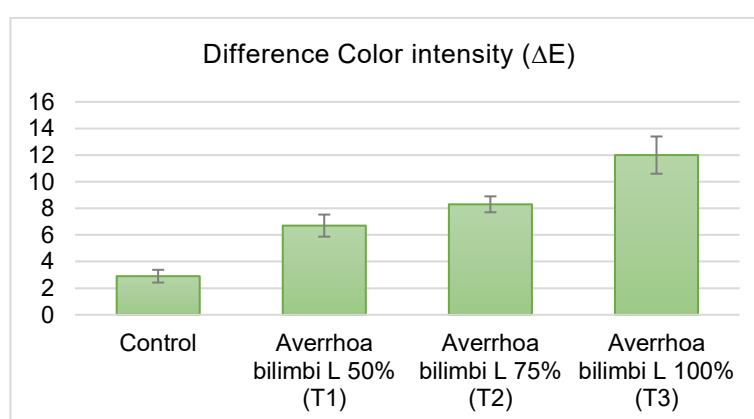


Figure 1. Graph of difference color intensity (ΔE) $\pm$ SD before and after treatment

Table 2. Post hoc Multiple Comparison test results of difference color intensity (ΔE)

Groups	Difference colour intensity (ΔE)			
	Control	T1	T2	T3

Control	0.000*	0.000*	0.000*
T1		0.035*	0.000*
T2			0.000*
T3			

*Significant difference (Post hoc Multiple Comparison :p<0.05)

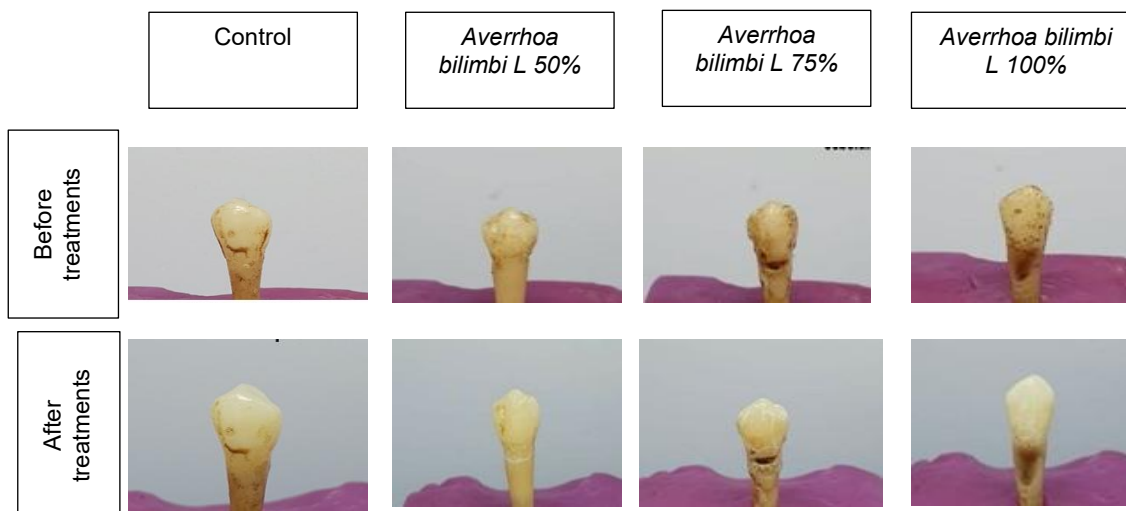


Figure 2. Condition of teeth before and after treatment at each concentration

DISCUSSION

Extrinsic dental stains or extrinsic decolorization are located on the outer surface of the teeth, and it attached to the teeth pellicle topically or through extrinsic agents^{11 14}. Pellicles have a tendency to facilitate the formation of extrinsic stains, especially in the difficult areas were not can be reached by tooth brushes and the abrasive action of toothpaste¹⁵. Extrinsic decolorization can occur through attachment of color pigments on the pellicle to produce stains or chemical interactions mechanism on the surface of the teeth¹⁶. Extrinsic stain models in this study was made by soaking teeth in robusta coffee solution for 14 days at room temperature. Previously teeth were soaked in artificial saliva for 2 minutes to facilitate an atmosphere in oral cavity. There is selective adhesion of positive ions from coffee to the tooth surface when pellicle and chromogen attachment from coffee to the tooth surface was happening. The tannin content in coffee is the cause of brownish discoloration on teeth, beside that tannin can reduce the pH of coffee so that the atmosphere becomes acidic which causes the enamel to become softer so it susceptible to stain infiltration¹⁷.

The image results were analyzed to obtain L, a, and b values using *Adobe Photoshop CC2018* software. Color measurement using L*, a* and b* value parameters in the CIE Lab system. Lab, a color model was designed to resemble the perception of human vision using three components, namely L as luminance (lighting) also a and b as color dimensions that opposite. The L* value is an important factor to determining the degree of brightness of tooth color before and after treatment. The value of L* represents the value or brightness level of an object and it is judged based on a set color scale, where L* 0 represents black while L* 100 is white, so that the higher of the L* value is the brighter tooth¹⁸.

Averrhoa bilimbi L extract was made by maceration method, it is simple and it can prevent thermolabile compounds¹⁹. Extraction of *Averrhoa bilimbi* L used 70% ethanol solvent because it is a universal solvent and it can dissolve almost all of organic compounds both polar and nonpolar^{16 20}. Treatment was carried out for 28 hours, this refers to Fauziah research in 2012 which states that *Averrhoa bilimbi* L extract gel in concentration 100% effective as an alternative bleaching ingredient by applied it on teeth 2 hours per day and repeated for 14 days⁸. The calculation of treatment time accumulation was previously carried out by Lumuhu's research in 2016 entitled the difference of the effectiveness of tomato and apple juice as natural ingredients for teeth whitening. It was done for 5 days or 120 hours with tooth color measurements on days 1, 3 and 5. It is refers to Meizarini's in 2005, she stated that carbamide peroxide 10%-22% effective in whitening teeth for 3-4 weeks with the use of 2-4 hours per day, it equivalent with 5 days with 24 hours use^{21,22}.

The measurement of difference color intensity (ΔE) is carried out by the formula of *CIELab with CIEDE2000 method*. It showed that the increasing of the L values occurred in all sample groups, this means that there were an increasing brightness values of the teeth to be whiter in each group. *The One-Way Anova* test showed $p < 0.05$, it means that the difference color intensity (ΔE) value in each group has a significant difference. Successive difference color intensity (ΔE) values ranging from smallest to largest, were in the salivary control group followed by *the Averrhoa bilimbi* L extract group of 50%, 75% and 100%. *The Post Hoc Multiple Comparison* test showed $p < 0.05$, it means that there were significant differences between each treatment group. The results of this study showed that *Averrhoa bilimbi* L extract solution began to work effectively at a concentration of 50%, because its difference color intensity (ΔE) was significantly different from the control group. The values of difference color intensity (ΔE) increase along with increasing concentrations of *Averrhoa bilimbi* L extract solution.

Oxalic acid has an important role in the discoloration of tooth samples with extrinsic stain soaked in *Averrhoa bilimbi* L extract solution, it was acts as an oxidizer. In 100 grams of *Averrhoa bilimbi* L has an oxalic acid content of 5.5-8.9%²³. It was thought able to whiten teeth and remove or fade extrinsic dental stains. Oxalic acid as an oxidizing agent will oxidize the stain on the surface of the tooth, the oxidation results will produce peroxide compounds which diffuse into tooth enamel and produces free radicals. They will react with stain molecules, undergo an oxidation process, it will dissolve the stain molecules on the teeth so that the extrinsic stain will disappear or fade^{18 24 25}. Slight color that occurred in the control group could happen because the presence of water content in saliva that could serves as *self-cleansing*²⁶. However, there are limitations in this study, that is the lowest concentration of *Averrhoa bilimbi* L extract solution is only up to a concentration of 50%, so it is not yet possible to know the minimum concentration that is effective for removing tooth extrinsic stains.

CONCLUSION

Averrhoa bilimbi L extract solution is effective to remove tooth extrinsic stains; it began to work effectively at a concentration of 50% and had the best results at a concentration of 100%. Comparing the different smaller doses and observation times, as well as toxicity and sensitivity tests, is necessary for further research.

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