THE COLOR CHANGE EFFECT OF THERMOPLASTIC NYLON AFTER IMMERSION IN 30% KELAKAI (*Stenochlaena palustris*) LEAF EXTRACT

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ABSTRACT
Background: *The most commonly used denture cleanser is alkaline peroxide, but this type of denture cleanser can cause color changes of the denture base and is relatively expensive, so alternative denture cleansers from natural ingredients are needed. Kelakai leaves have an antifungal property that can be used as a natural-based denture cleanser.* **Purpose:** *To determine the color change effect of the thermoplastic nylon after immersion in 30% kelakai leaves extract.* **Methods:** *This study used a true experimental method with pre-test and post-test and control group design. The research used 24 round-shaped samples with 20 mm diameters and 3 mm thickness. There were 3 groups of immersion, including 30% kelakai leaf extract, alkaline peroxide, and distilled water.* **Results:** *The results showed the mean of color changes score for kelakai leaf extract (3,71), alkaline peroxide (2,59), and sterile distilled water (2,29). Data were analyzed using One Way ANOVA and Post Hoc Bonferroni.* **Conclusion:** *It can be concluded that there is an effect on color change of thermoplastic nylon immersed in 30% kelakai leaf extract, alkaline peroxide, and sterile distilled water. The highest to the lowest mean of color changes score was 30% kelakai leaf extract, alkaline peroxide, and distilled water.*

Keywords: Color changes, kelakai leaf extract 30%, thermoplastic nylon
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INTRODUCTION

Tooth loss can be treated by making dentures.[1] The use of dentures to replace missing teeth is very important to restore masticatory function, speech function, and aesthetic function. Dentures are prostheses that replace partially or completely missing teeth and the surrounding tissue.[2] According to RISKESDAS 2018, the national prevalence rate of the Indonesian population experiencing tooth loss is 19%, while in South Kalimantan, it is 17,8%. The highest percentage of the Indonesian population who performs denture installation is in South Kalimantan Province (3,3%), which is higher than the average denture installation in Indonesia (1,4%). The percentage of denture installation is dominated by the age 65 years old and over (4,1%).[3]

The most common denture base material is acrylic resin, but this material breaks easily and can cause allergies. This makes thermoplastic resins became an alternative to acrylic resins.[4] There are four types of thermoplastic resins: acetal thermoplastic, polycarbonate thermoplastic, acrylic thermoplastic, and nylon thermoplastic. The earliest thermoplastic resins introduced were thermoplastic nylon or polyamide.[5] Thermoplastic nylon became the choice because it has excellent aesthetics due to its transparent color so that it can resemble mucosa and is free from residual monomers so that it is biocompatible for denture users. This type of resin also has high flexibility, good heat resistance, non-porous, and resistant to chemicals, but still has disadvantages such as high water absorption and low color stability.[6]

Thermoplastic nylon content that can affect the occurrence of water absorption is amide compounds. The more amide compounds, the greater water absorption occurs. Water absorption is the process of water molecules diffusion into the polymer chain which will affect the chemical structure of a material. The incoming water

molecules will separate the chain bonds, then occupy the intermolecular space to stretch and the rate of water absorption becomes higher. High water absorption will affect the material properties of the denture base such as color stability.[6,7]

Color stability is the ability of a material or surface layer to maintain its color from environmental degradation.[8] Color change can be measured using digital analysis tools with the CIELab (Commission Internationale de L'Eclairage) system recommended by ADA (American Dental Association). The CIELab system uses three color coordinates L, a, and b.[9] This system is a development of the Munsell method which consists of three basic color spaces known as hue, value, and chroma.[10] The color change value is written with the symbol E. If $\Delta E < 1$ the color change is not visible to the human eye, $1 < \Delta E < 3,7$ the color change is clinically acceptable, and $\Delta E > 3,7$ the color change is very obvious and clinically unacceptable.[11] Color changes occur due to two factors: intrinsic and extrinsic factors. An intrinsic factor is a color change that occurs due to a change in the chemical structure of the material, for example, the oxidation of amine compounds. An extrinsic factor is discolorations that occur due to contact with external dyes, such as colored drinks and denture cleaning agents.[4]

The denture base needs to be cleaned to prevent a fungal infection of Candida albicans. Candida albicans can adhere to the surface of the denture base, then release products in the form of endotoxins that can damage oral mucosa and cause denture stomatitis.[12] Cleaning dentures is divided into three methods based on how it works: mechanically, chemically, and combination of both methods. Mechanical cleaning is carried out with a soft-bristled toothbrush using liquid soap, water, paste or powder, and an ultrasonic cleaning device.[13] Chemical cleaning is carried out by immersing the denture in a disinfectant solution, denture cleanser, oxygenating agents, and microwave radiation.[14] Combined cleaning methods can be done in two ways by immersing the denture in a denture cleanser and vibrating it with an ultrasonic device or brushing the denture first and then soaking it with a disinfectant solution.[13] Kumar et al (2017) recommend chemical cleaning of dentures using the immersion method in elderly patients due to lack of hand dexterity with age. This method is also easy to use and can reach the undercut of the denture base.[15]

Denture cleaning can be done using a denture cleanser. The most commonly used denture cleanser is alkaline peroxide. This type of denture cleanser is relatively expensive and can fade the denture base color so that alternative denture cleanser from natural ingredients is needed to overcome this problem.[16] 30% kelakai leaves have the same ability as alkaline peroxide on inhibiting the growth of Candida albicans and have an inhibition zone that is strong-categorized as an antimicrobial substance. Based on this background, research is needed on the immersion effect of 30% kelakai leaf extract (*Stenochlaena palustris*) on thermoplastic nylon color changes score.

MATERIAL AND METHODS

This study used a true experimental method with pre-test and post-test and control group design. This research has been declared ethically proper with No. 020/KEPKG-FKGULM/EC/II/2021. The research used 24 round-shaped samples with 20 mm diameters and 3 mm thickness which were selected using a simple random sampling technique as many as 8 nylon thermoplastic plates in each immersion group. There were 3 groups of immersion, including 30% kelakai leaf extract, alkaline peroxide, and distilled water. Samples were immersed for 3 days 19 hours 15 minutes. The sample's inclusion criteria were nylon thermoplastic resin plate which was cylindrical, 20 mm in diameters, 3 mm thick, flat, smooth, and non-porous surface. The sample's exclusion criteria included broken or scratched nylon thermoplastic resin plates, contaminated with other materials or dirty, and those with discoloration.

Thermoplastic Nylon Manufacturing

Samples were made using the injection moulding technique, which began with making molds of cylindrical acrylic glass with 20 ± 1 mm diameters and 3 ± 1 mm thickness and making a sprue from base plate wax. The cuvette was covered with vaseline and filled with a type 3 plaster cast which had been made according to the manufacturer's regulations. Acrylic glass was embedded in a cuvette which has been filled with cast and sprue was attached from the back of the cuvette to the posterior part of the wax model. The plaster was trimmed and smeared with vaseline after hardens. The antagonist cuvette was installed and filled with type 3 plaster, closed and pressed with a press for ±30 minutes. The cuvette was immersed in boiling water for dewaxing, then the cuvette was opened. The acrylic glass was removed and the cuvette was cleaned of the remaining red wax.

The cleaned mold space was covered with vaseline, the thermoplastic nylon was inserted into the cartridge and melted at a temperature of $\pm280^{o}C$ using an electric furnace for 11 minutes. The melted thermoplastic nylon was injected into

the mold space with a plunger under a hydraulic press with a pressure of 6-8 bars for 5 minutes. The cuvette was removed and allowed to cool at room temperature for 30 minutes. After that, the cuvette was opened and the thermoplastic nylon plate was removed.

The polishing procedure was carried out by one person to standardize the surface roughness so that the pressure applied was the same and stable so that the same surface roughness was obtained for each sample. The sharp parts of the thermoplastic nylon plate were trimmed using a frasser bur, the placed on a rotary grinder coated with 400, 800, and 1200 sandpaper while running with water for 5 minutes at a speed of 500 rpm. The sample was polished using a polishing motor and a cotton wheel bur with pumice to obtain a smooth sample surface.[17,18]

Kelakai Leaf Maceration

Kelakai leaf extract was prepared by the maceration method. The leaf criteria were young leaves, light green, clean, fresh, not perforated, not damaged, not affected by pests and fungi, picked by hand in the morning, and taken in Anjir, Barito Kuala. 2 Kg of kelakai leaves placed in a container equipped with a cooler to maintain the freshness of the leaves. The kelakai leaves were washed, then dried in an oven at $40^{o}C$ for 4 hours. Kelakai leaves were cut into small pieces, then the pieces of the leaves were mashed using a blender and filtered until the leaves simplicia were obtained.

The leaves simplicia was added with 1000 ml of 96% ethanol solvent, the process was carried out for 24 hours while stirring using a shaker. The extract was filtered using filter paper, then the filtrate was evaporated using a rotary evaporator at a temperature of 40-$50^{o}C$ for 4-6 hours, then heated using a water bath to obtain 100% kelakai leaf concentrated extract. 30% kelakai leaf extract was obtained by diluting 15 ml of the 100% kelakai leaf concentrated extract and 35 ml of distilled water.[19,20]

Color Changes Measuring

Measurement of color change was carried out before and after immersion of the sample with 30% kelakai leaf extract. Before measuring the color change, the samples were immersed in a saline solution and incubated using an incubator at $37^{o}C$ for 24 hours. The samples were divided into 3 groups, each group consisted of 8 samples.[21] The first group samples were immersed in 30% kelakai leaf extract, the second group samples were immersed in alkaline peroxide, and the third group samples were immersed in sterile distilled water. All immersion groups were immersed for 3 days 19 hours 15 minutes which was equivalent to immersing the denture base for 15 minutes every day for 12 months.

Color changes were measured using digital analysis tools with the CIELab system. The sample was put into a mini studio photo box. The distance between the camera lens and the sample was 40 cm. The sample was placed in the center and adjusted to its proper position. The image was taken with a webcam and saved in JPG format. Color changes were detected by a special application using the CIELab color standard. The color change value was obtained by calculation using this equation.

$$\Delta E=[(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{1/2}$$

Notes:
L: Black-white coordinates (0 to 100)
a: Green-red coordinates (-120 to +120)
b: Blue-yellow coordinates (-120 to +120)
L_t, a_t, b_t: Before immersion
L_o, a_o, b_o: After immersion
$\Delta L: L_o - L_t$
$\Delta a: a_o - a_t$
$\Delta b: b_o - b_t$

The value of ΔE is converted into three values:
a. $\Delta E<1$: color change is not visible to the human eye
b. $1<\Delta E<3,7$: clinically acceptable color change
c. $\Delta E>3,7$: color change is very obvious and clinically unacceptable.[11]

RESULTS

Based on measurements that have been done, the following average values of color change were obtained.

Table 1. Mean value and standard deviation of the color change value on thermoplastic nylon

Immersion Groups	Mean ± SD
Kelakai leaf extract 30%	3,71 ± 1,12
Alkaline peroxide	2,59 ± 0,86
Sterile distilled water	2,29 ± 0,67

The results showed that the lowest mean value of color change was found in sterile distilled water group immersion, while the highest mean value was found in 30% kelakai leaf extract immersion. If three groups were compared, the average value of color change in the 30% kelakai leaf extract immersion was higher than alkaline peroxide and sterile distilled water immersion groups. The data showed the value of $\Delta E > 3,7$ which means that the color change was visible and clinically unacceptable, while the immersion of the sample in alkaline peroxide and sterile distilled

water $\Delta E < 3,7$ so it can be interpreted that the color change is clinically acceptable.

Table 2.   Value of ΔL, Δa, and Δb

Immersion Groups	ΔL	Δa	Δb
Kelakai leaf extract 30%	0,25	-2,38	0,75
Alkaline peroxide	0,13	0,38	1,88
Sterile distilled water	0,75	0,38	-1,5

The ΔL value of the three treatment groups is positive, which means there is an increase in brightness. The Δa value of the extract of the leaves is negative, which means the color is more greenish, while the Δa value of alkaline peroxide and distilled water is positive, which means the color is more reddish. The value of Δb for 30% kelakai leaf extract and alkaline peroxide is positive, which means the color is positive, which means the color is more yellowish, while the value of Δb for sterile distilled water is negative, which means the color is more bluish.

The data were tested for normality with Shapiro-Wilk and the results obtained were $p=0,314$ in the alkaline peroxide group and $p=0,379$ in the sterile distilled water group. The data showed a value of $p>0,05$ which meant the data was normally distributed. Then the Levene's test homogeneity test was carried out and the p-value = 0,569 which meant $p>0,05$, so it can be concluded that the data variance is homogeneous. The test results met the requirements for the One Way ANOVA parametric test.

The results of the One Way ANOVA test showed $p=0,012$ ($p<0,05$) which means there is a significant difference at least in one immersion group. To find out which group had a significant difference, it was continued with the Post Hoc Bonferroni test.

Table 3.   Post Hoc Bonferroni test result

	P-value		
	Kelakai leaf extract 30%	Alkaline peroxide	Sterile distilled water
Kelakai leaf extract 30%	_____	0,06	0,01*
Alkaline peroxide	0,06	_____	1,00
Sterile distilled water	0,01*	1,00	_____

* = there was a significant difference ($p<0,05$) using Post Hoc Bonferroni test

The results of the Post Hoc Bonferroni test showed that there was a significant difference ($p<0,05$) between the 30% kelakai leaf extract group compared to the sterile distilled water group.

DISCUSSION

Based on the results of this study, there was an effect on color change value of thermoplastic nylon immersed in 30% kelakai leaf extract. Based on the average values of ΔL, Δa, dan Δb in table 2, the ΔL value of the three immersion groups are positive, which means that there is an increase in brightness. The value of Δa in the sterile distilled water and alkaline peroxide immersion group was positive so that the color became more reddish, while the Δa value in the 30% kelakai leaf extract immersion group was negative so that the color became greener. The value of Δb in the sterile distilled water immersion group was negative so that the color became more bluish, while the b value in the alkaline peroxide immersion group and 30% kelakai leaf extract was positive so that the color became more yellowish.

The results of color change in this study is in line with the study conducted by Hanifa et al (2018), which stated that sterile distilled water and alkaline peroxide immersion groups experience increased ΔL value (black-white) and Δa value (green-red) so that in these immersion groups have more faded and reddish color. Δb value (blue-yellow) in sterile distilled water immersion group experienced decreased so that it causes the color to be bluer, while in alkaline peroxide immersion group experiences increased so that its color becomes yellower.[9]

The results of this study showed that the color change value in the 30% kelakai leaf extract immersion group is higher than the sterile distilled water immersion group. This is in line with the study conducted by Wibawaningtyas (2017), which showed that the results of distilled water immersion group have a smaller color change value than the extract immersion group.[22] The results of the study showed that the color change value in the alkaline peroxide immersion group is higher than the sterile distilled water immersion group. This is in line with a study by Awing and Koyama (2013) and Helaly et al (2018), which also showed that color change value in the alkaline peroxide immersion group is higher than sterile distilled water immersion group.[4,23]

Color changes occur due to two factors, intrinsic and extrinsic factors. Intrinsic factor is a change in the chemical structure of the material, while extrinsic factors such as contact with external coloring agents such as colored drinks and denture cleaning agents.[4] Other factors that play a role in color changes are water absorption, surface roughness, microporosity, and prolonged contact with the color substance. Water absorption plays a very important role in the occurrence of color changes in thermoplastic nylon because this material is hygroscopic or easily absorbs water.

Surface roughness will affect the color of a material. The rougher it is, the easier it is for stains to accumulate on a material, causing color changes. Microporosity can cause color particles to stick to porous areas. The more porosity, the more accumulation of color substance that is absorbed through the diffusion process. The length of contact with the color substance will affect the color change in a material. The longer material is immersed in the color substance, the greater the color change occurs.[24]

The color change in the 30% kelakai leaf extract immersion group had a higher value than the alkaline peroxide and sterile distilled water immersion group. This is because the extract contains flavonoids. Flavonoids are active phenolic compounds, which can cause color changes of the denture base by removing the H+ bond so that the C and O double bonds are broken. The C atom is more strongly bound to O in the phenol polymer chain (C_6H_5OH) so that the H ion is easily oxidized. Oxidation of H ions will cause the release of phenoxide anions and H+ cations. The H+ cation binds to the O atom in the polyamide chain ($NH_2(CH_2)_6NHCO(CH_2)_6COOH$) causing color changes in thermoplastic nylon.[5,7]

The color change that occurred in the alkaline peroxide immersion group was caused by the presence of sodium perborate. This content will decompose and form hydrogen peroxide when dissolved in water. This compound will release oxygen which is a bleaching agent. The process of decomposition of hydrogen peroxide into oxygen and water occurs very slowly. The perhydroxyl anion which is the active component of hydrogen peroxide is formed through the ionization of H_2O_2 which reacts with the chromophore of the material through oxidation, this can cause a material to change color.[4] The immersion group with sterile distilled water had the lowest average value of color change because sterile distilled water was pure water that only contained H_2O without any additional ions. The content in this sterile distilled water is colorless, odorless, and has no taste.[25] Based on the results of the study, it can be concluded that there is an effect on the color change of thermoplastic nylon immersed in 30% kelakai leaf extract, alkaline peroxide, and sterile distilled water.

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