

**DENTINO**  
**JURNAL KEDOKTERAN GIGI**  
**Vol VII. No 2. September 2022**

**THE IMMERSION EFFECT OF 30% *Stenochlaena palustris* LEAVES EXTRACT ON DISCOLORATION OF HEAT CURED ACRYLIC RESIN BASE**

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**ABSTRACT**

**Background:** South Kalimantan Province is one of the regions in Indonesia with the highest use of dentures with a percentage of 3.3%. Dentures should be cleaned frequently to help maintain their quality, particularly using a denture cleanser. The most widely-used denture cleanser in the community is alkaline peroxide. Alkaline peroxide can effectively inhibit the growth of *Candida albicans* on dentures. However, long-term use of alkaline peroxide may lead to discoloration of the acrylic resin denture base. The 30% kelakai leaves extract may be used as an alternative to denture cleanser, because it is recognized to have antifungal properties and is expected to be capable of minimizing the effect of discoloration. **Objective:** To analyze the immersion effect of 30% kelakai leaves extract on discoloration of heat cured acrylic resin base. **Methods:** This research was a true experiment with a pretest and posttest design with a control group using a heat cured acrylic resin sample with a diameter of 15mm x 2mm thick. A total of 21 samples were divided into 3 groups of immersion, namely 30% kelakai leaves extract, alkaline peroxide, and aquadest. Samples were measured before and after immersion for 5 days to assess discoloration using a series of digital analysis tools and the CIELAB system. The value obtained is entered in the equation  $\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$ . **Results:** One Way ANOVA test and Post Hoc Bonferroni statistical test showed that there was a significant difference ( $p < 0.05$ ) between the 30% kelakai leaves extract ( $7.04 \pm 0.31$ ), alkaline peroxide ( $3.01 \pm 0.29$ ), and aquadest ( $2.31 \pm 0.63$ ). Each immersion group indicated significantly different results. **Conclusion:** There was a change in the color of the heat cured acrylic resin base after immersion in 30% kelakai leaf extract.

**Keywords:** Discoloration, Heat cured acrylic resin, Digital analysis, *Stenochlaena palustris* leaf, System CIELAB.

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**INTRODUCTION**

South Kalimantan Province is one of the regions in Indonesia with the highest use of dentures with a percentage of 3.3%.<sup>1</sup> Dentures are one of the treatments used to replace missing teeth and to improve masticatory, phonetic, and aesthetic functions.<sup>2</sup> Complete dentures are typically composed of artificial teeth and denture bases.<sup>3</sup> The most frequently used base materials in the fabrication of denture bases are heat-cured acrylic resins, because they have good esthetics, are gum-colored, easy to manipulate, economical, and can be repaired.<sup>2,4</sup> However, heat-cured acrylic resin has certain disadvantages such as porosity and water absorption ability which can affect the discoloration over a period of time.<sup>5,6,7,8</sup> The discoloration may be

caused by the denture cleanser through the diffusion mechanism of water molecules which may generate a discoloration by degradation of the polymer chain which subsequently lead to the separation of the acrylic resin monomer.<sup>8,9,10</sup>

The use of acrylic resin dentures for a long period of time is able to enhance the growth of *Candida albicans* which causes denture stomatitis.<sup>5</sup> Denture stomatitis is an oral cavity lesion that is often found in denture users with a fairly high prevalence in a range between 30%-50%.<sup>6</sup> Denture stomatitis could be minimized by cleaning the denture. Denture cleaning is mechanically performed by utilizing a toothbrush, and chemically using a denture cleanser. Moreover, a combined

denture-cleaning method is considered as a combination of mechanical and chemical methods using a toothbrush and a denture cleanser. One of the denture cleansers commonly used by the community is alkaline peroxide.<sup>2,11</sup>

Soaking the denture in alkaline peroxide for 20 minutes may effectively inhibit the growth of *Candida albicans*.<sup>7</sup> Alkaline peroxide as a denture cleanser has certain disadvantages, including chemical-based, expensive, difficult to obtain due to its classification as imported goods, and may lead to discoloration of acrylic resin when used for a continuous period of time.<sup>6,7,8</sup>

An alternative plant for the natural denture cleanser used in this research was kelakai leaves (*Stenochlaena palustris* (Burm.) Bedd.). Kelakai leaves are one of the traditional plants that are frequently found in peat swamp areas such as South Kalimantan. Kelakai leaves may effectively inhibit the growth of *Candida albicans* at a concentration of 30% with a diameter of  $16.60 \pm 0.45$  and classified in the strong category.<sup>12</sup> Based on Arullappan et al (2017), the largest content in the leaves of kelakai is flavonoids (503.56 mg QE/g), which may function as antifungals by binding to the cell wall through a protein-phenol complex which leads the intracellular matrix of the fungus to exit and results in cell death.<sup>13,14</sup> Flavonoid compounds contribute to acidic properties that may affect the physical properties of acrylic resins, including discoloration when in contact with acrylic resins.<sup>6</sup> Referring to the description above, it is necessary to conduct a study to determine the presence or absence of color changes after immersion of a heat-cured acrylic resin base on 30% kelakai leaves extract.

## RESEARCH METHODS

This research was a true experimental study with a pretest and posttest research design with a control group which has been declared ethically feasible by the Health Research Ethics Commission, Faculty of Dentistry, Lambung Mangkurat University with No. 019/KEPKG-FKGULM/EC/II/2021. The total samples of heat cured acrylic resin used in this research were 18 pieces with sizes according to ADA specifications no. 17 diameter 15mm x 2mm cylindrical in shape. The inclusion criteria for the sample are smooth, non-porous, and glossy surface; while the exclusion criteria were damaged and broken samples. The sampling technique used simple random sampling which was divided into 3 treatment groups, namely: the 30% kelakai leaves extract group, the alkaline peroxide group, and the aquadest group. Acrylic resin immersion was carried out for 5 days with a simulated denture soaking for 20 minutes per day for 1 year.

## Heat Cured Acrylic Resin

Heat-cured acrylic resin samples were created at Arsynonsne Dental Surabaya. In the first stage, a vertex mold was made with a size of 15mm x 2mm. Furthermore, the type III gypsum dough was mixed using a bowl and a spatula until a homogeneous solution was obtained. The ready dough was then poured into the bottom cuvette and was vibrated to remove air bubbles. The gypsum surface that had been set was subsequently smeared with vaseline evenly. The vertex mold was placed on top of the gypsum, the top cuvette was installed and the dough was poured back until the cuvette was full. After the vertex mold setting was removed, the polymer and monomer of heat-cured acrylic resin were mixed using a stelon pot and a cement spatula in a ratio of 3:1 until the dough stage was reached. The dough was placed in a mold space that had been smeared with CMS. Plastic cellophane was placed between the top and bottom cuvettes to be further pressed using a hydraulic press with a pressure of 1000 psi, the cuvette was opened and the excess acrylic was cut. The cuvette was closed again and was re-pressured by 2200 psi. Subsequently, the cuvette was pressed using a hand press and boiled for 30 minutes. The acrylic resin samples were removed and continued in the finishing and polishing process using a Fraser bur, stone bur, cotton wheel bur, and 600 and 1200 sandpaper. Fraser burs were used to remove sharp parts, while stone burs and sandpaper were used for smoothing surfaces, and cotton wheel burs with pumice were utilized for polishing acrylic resin.

## Kelakai Leaves Extract

The kelakai leaves extract was made at the Biochemistry Laboratory, Faculty of Medicine, Lambung Mangkurat University. Fresh and fully expanded mature green leaves of kelakai amounted to 1 kg were selected in this research. The kelakai leaves were cleaned, cut, and dried at room temperature. Kelakai leaves were put in the oven for 4 hours at 40°C and then blended until the simplicia powder was obtained. The maceration process was successfully carried out by inserting the kelakai leaves powder in the extractor and adding 1 litre of 96% ethanol in a period of 1x24 hours. This was intended to determine the active compounds contained in the leaves of kelakai which were dissolved in 96% ethanol. Subsequently, the solvent was evaporated using a vacuum rotatory evaporator at a temperature of 50-60°C for 4-6 hours, and the solvent was heated over a water bath until all the solvent evaporated and a brown residue was obtained as the main solution with a concentration of 100%. Ethanol-free test was carried out by adding potassium dichromate ( $K_2Cr_2O_7$ ) to the extract. If discoloration was not occurring, no ethanol content was detected in the extract. The

main solution was dissolved in DMSO in a ratio of 1:1. Furthermore, the dilution was carried out until the extract was obtained with a concentration of 30%.

#### Discoloration Measurements

Measurement of discoloration was carried out at the Biochemistry Laboratory, Faculty of Medicine, Lambung Mangkurat University. Acrylic resin samples were incubated with saline solution at 37°C at a time period of 1x24 hours in order to adjust to the conditions of the oral cavity. The color measurement of the sample was successfully carried prior to the immersion process (pretest) using a series of digital analysis tools. Acrylic samples were put in a dark box with standardized LED lights. The pictures were taken using microsoft lifecam studio and saved in JPG format. The distance between microsoft lifecam studio and the sample was 40 cm. Images were then converted to numbers to be compared with the MATLAB application. Subsequently, the samples were immersed in 3 groups: 30% extract of kelakai leaves, alkaline peroxide (positive control), and distilled water (negative control) for 5 days. Furthermore, the color of the sample after immersion (post-test) was measured using a series of digital analysis tools. Discoloration was detected using the color system standard recommended by the ADA, namely CIELAB. The discoloration of each sample was successfully calculated by the following equation:

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

$$\Delta L = L_o - L_t$$

$$\Delta a = a_o - a_t$$

$$\Delta b = b_o - b_t$$

Information:

- $\Delta E$  : Total Color Change.
- $L$  : Brightness coordinates, 0 (black) to 100 (white).
- $a$  : Green-red color coordinates.
- $b$  : Blue-yellow color coordinates.
- $L_t, a_t, b_t$  : Before immersion.
- $L_o, a_o, b_o$  : After immersion.

After obtaining the discoloration value, the data were statistically tested with the Shapiro-wilk test to determine the normality of the data and Levene's test to determine the data homogeneity. Furthermore, parametric analysis was performed using the One-Way ANOVA hypothesis test and continued with the Post Hoc Bonferroni test. The data were then processed using SPSS v.26.

## RESULTS

Regarding to the test of immersion effect of 30% kelakai leaves extract, it was obtained the results of the average value of the color change measurement which are presented in table 1.

**Table 1.** Average color change value of heat cured acrylic resin and standard deviation

Immersion Group	Mean $\pm$ SD
30% Kelakai Leaves Extract	7,04 $\pm$ 0,31
Alkaline Peroxide	3,01 $\pm$ 0,29
Aquadest	2,31 $\pm$ 0,63

According to table 1, it was found that the highest average value of heat-cured acrylic resin discoloration value occurred in the group of 30% kelakai leaves extract (7.04), while the lowest average value of heat-cured acrylic resin discoloration occurred in the aquadest solution immersion group (2.31).

**Table 2.** Average Color Change Value of Heat Cured Acrylic Resin Based on  $\Delta L$ ,  $\Delta a$ , dan  $\Delta b$ .

Immersion Group	$\Delta L$	$\Delta a$	$\Delta b$
30% Kelakai Leaves Extract	2,43	-4,14	4,29
Alkaline Peroxide	1,14	0,71	0,71
Aquadest	0,86	0,71	-0,57

Referring to table 2, it was indicated that all immersion groups obtained  $\Delta L$  (+). This indicates that the intensity of the brightness on the sample was brighter. The immersion group in alkaline peroxide solution and aquadest solution obtained  $\Delta a$  (+), which defined that the color intensity in the sample was redder, while the immersion group in the 30% extract of the kelakai leaves obtained  $\Delta a$  (-), which indicated that the color intensity in the sample was greener. The immersion group of 30% extract of the kelakai leaves and alkaline peroxide solution obtained  $\Delta b$  (+), which indicated that the color intensity of the sample was more yellow, while the aquadest solution immersion group obtained  $\Delta b$  (-), which indicated that the color intensity of the sample was bluer.

The results of testing the effect of immersing 30% extract of the kelakai leaves on the discoloration of heat-cured acrylic resin base were processed by utilizing IBM SPSS Statistics version

26.0. The data normality test was carried out using the Shapiro Wilk test and the data homogeneity test was performed using Levene's test. The results of all groups showed that the data were normally distributed ( $p > 0.05$ ) and the data variance was homogeneous ( $p > 0.05$ ). Moreover, it was continued to conduct the comparative hypothesis test  $> 2$  parametric groups, specifically the One Way ANOVA test. The results of the One-Way ANOVA statistical test showed a value of  $p = 0.000$  ( $p < 0.05$ ), which means that there was a significant difference between the treatment groups.

**Table 3.** Bonferroni Post Hoc Test Results

	P Value		
	EDK 30%	Alkaline Peroxide	Aquadest
EDK 30%	_____	0,00*	0,00*
Alkaline Peroxide	_____	_____	0,02*
Aquadest	_____	_____	_____

EDK: 30% Kelakai Leaves Extract

\* There are significant differences ( $p < 0,05$ )

In regards to table 3, the results of the Post Hoc Bonferroni test showed that there was a significant difference ( $p < 0.05$ ) between the immersion group of 30% extract of kelakai leaves and the alkaline peroxide immersion group, between the immersion group of 30% extract of kelakai leaves and the aquadest immersion group, and between the alkaline peroxide immersion group and the aquadest immersion group.

## DISCUSSION

The discoloration of acrylic resin was influenced by several factors, specifically the ability of liquid absorption in the material, the ratio of unsuitable polymer monomer which results in microporosity, sample size, and duration of contact.<sup>8</sup> The longer the contact duration of acrylic resin immersed in a solution, the greater the discoloration detected.<sup>10,15</sup>

Samples of heat-cured acrylic resin immersed in 30% extract of kelakai leaves solution for 5 days showed an increase in the value of L (2.43), a decrease in the value of a (-4.14), and an increase in the value of b (4.29), so that the results indicated the lighter, green, yellowish colors. The discoloration to a lighter color was caused by the diluent used to make the 30% extract of kelakai leaves, particularly aquadest. Aquadest penetrates into the resin matrix and leads to the formation of porosity and discoloration to a lighter color.<sup>16,17</sup>

Acrylic resin which was immersed in 30% extract of kelakai leaves also decreased at a value of -4.14 and became greener. This change was influenced by the chlorophyll compounds in the leaves of kelakai.<sup>13</sup> Chlorophyll is a secondary metabolite compound in all green plants and is able to produce green pigments.<sup>18,19</sup>

The highest discoloration value was found in the value of b which was amounted to 4.29 that leads to a more yellowish acrylic resin. This change was due to the highest content in the extract of kelakai leaves, particularly flavonoids (503.56 mg QE/g).<sup>8,17</sup> According to Syamsul (2019), most of the flavonoids are yellow pigments. Flavonoids have a chromophore group which is able to produce a color pigment and an auxochrome group which functions to bind the color which may result in discoloration of the acrylic resin which becomes more yellowish.<sup>7,20</sup> Flavonoids release H<sup>+</sup> ions and benzene groups when in contact with heat-cured acrylic resin, while the ester groups on acrylic resins will release CH<sub>3</sub>O<sup>-</sup> and RCO groups. The H<sup>+</sup> ion released from the flavonoid will bind to the CH<sub>3</sub>O<sup>-</sup> released from the ester group, while the benzene group on the flavonoid will bind to the RCO group of the ester group.<sup>5,6</sup> The bond formed from a polymer chain with a phenol group is a C=O double bond.<sup>6,21</sup> These bonds will penetrate the mass of polymethyl methacrylate, resulting in a decrease in the strength of the polymer chain bonds and may lead to chemical damage. The chemical damage may affect the discoloration which is recognized as part of one of the acrylic resin properties, namely color stability.<sup>5,22</sup>

Discoloration on heat-cured acrylic resin immersed in alkaline peroxide solution showed an increase in the value of L (1.14), an increase in the value of a (0.71), and an increase in the value of b (0.71). The discoloration was predominately in a lighter or faded direction. These changes were caused by the sodium perborate compound in alkaline peroxide.<sup>23</sup> When the tablet is dissolved in water, the sodium perborate will decompose into alkaline peroxide. The alkaline peroxide reacts to produce H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) + alkali → 2H<sub>2</sub>O<sub>2</sub> + 2O (nascent oxygen).<sup>7,23</sup> Nascent oxygen has a very strong oxidative action. Therefore, the oxygen released may lead to the tertiary amine accelerator in the oxidized acrylic resin or the unreacted double bonds in the resin matrix. Consequently, this mechanism may increase the discoloration value of the heat-cured acrylic resin after the immersion.<sup>7,24,25</sup>

Acrylic resin immersed in aquadest solution showed an increase in the value of L (0.86), an increase in the value of a (0.71), and a decrease in the value of b (-0.57). The insignificant discoloration was caused by the content in the

distilled water which only consisted of pure water (H<sub>2</sub>O) and had no mineral content. Thus, the less particle fusion that occurred was also not able to accelerate the polymer chain degradation of the acrylic resin.<sup>7,8,16</sup> Referring to those matters, it may be inferred that the 30% extract of kelakai leaves could not be used as an alternative to natural denture cleansers in terms of the discoloration value of acrylic resin, which was higher than alkaline peroxide as a denture cleanser commonly used by the community.

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