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THE EFFECT OF IMMERSION IN 50% CONCENTRATION OF KASTURI LEAF EXTRACT (MANGIFERA CASTURI) ON THE DISCOLORATION OF HEAT CURED ACRYLIC RESIN

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ABSTRACT
Background: *Heat cured acrylic resin and alkaline proxide are the most used material for denture base and denture cleaner. However, the cost of denture cleanser and its discoloration effect after long term use became the major disadvantages of this material. Kasturi leaf extract, in 50% concentration, had been proven to have antibacterial and antifungal effect, such as a denture cleanser properties..* ***Objective:*** *This study was to determine the discoloration effect of immersing heat cured resin acrylic in to 50% Kasturi leaf extract solution compared to alkaline peroxide and distilled water.* ***Material and Methods:*** *This study used 24 samples of heat cured acrylic following ADA specification no 17, with 15 milimeters diameter and 2 millimeters of thickness. The 50% Kasturi leaf extract treatment group and the control group alkaline peroxide and aquades. Each group consisted of 8 samples which measured the discoloration before and after being immersed for 5 days. The discoloration of the sample was tested using a series of digital analysis tools. The results obtained then converted into the equation of* $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$. ***Results:*** *One Way ANOVA and Post Hoct Bonferroni statistical tests showed that there was a significant difference (p <0.05) between the immersion groups in 50% Kasturi leaf extract (6.75 ± 1.31), alkaline peroxide (3.29 ± 0, 57) and distilled water (2.04 ± 0.60).* ***Conclusion:*** *A 50% solution of Kasturi leaf extract has an effect on the discoloration of heat cured type acrylic resin.*

Keywords*: Discoloration, Heat cured acrylic resin, Kasturi leaves*
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INTRODUCTION

A good acrylic resin denture has a color like oral cavity tissue. One of the important factors for denture materials is color stability because discoloration is a sign of aging and denture damage. Denture replacement can be caused by discoloration. Denture replacement by discoloration results in losses for doctors and patients in terms of money and time.[1]

Color stability can be affected by changing the color of a material. There are two factors for the discoloration, internal and external. Internal factor includes the material of acrylic resin itself, either due to material properties, chemical structures, or monomer used, while external factor includes the absorption of dyes from external sources, such as coffee, natural dyes, or the denture cleaning agent.[2]

A denture cleaner can prevent the formation of dental plaque, remove food debris, tartar, and prevent the discoloration of denture. A dirty denture can cause bad breath, bad aesthetics, and inflammation of the oral mucosa, such as *denture stomatitis*. *Denture stomatitis* is caused by the infection of *Candida albicans* fungal on an untreated denture.[3] Denture can be cleaned mechanically, chemically, and a combination of both. Mechanical cleaning can be performed by brushing teeth using a paste, powder, or ultrasonic cleaner. The chemical cleaning method includes microwave radiation, oxygen exposure with air-drying, and immersion in the cleaning solution.[4]

Commonly available denture cleanser in the community are alkaline peroxide and sodium hypochlorite.[5] However, alkaline peroxide and sodium hypochlorite can cause whiteness on acrylic resin.[6,7] Until this time, denture cleaner circulating in the community still has a high price and imported so that it is difficult to obtain. Therefore, alternative

material that can be used as denture cleaner is required.[8]

One of the alternative materials from Indonesian plants that can be used as denture cleaner is Kasturi leaves. Kasturi is known can be used as a traditional medicine because it contains flavonoids and phenolic compounds. Based on the study by Marliani 2016, Kasturi leaves contain phenolics of 18.44% and flavonoids of 9.27% higher than the content in the bark and rind of Kasturi.[9] Kasturi leaves at concentrations of 25% to 100% have an inhibition zone against *Candida albicans*. In the previous study, it was proven that Kasturi extracts at a concentration of 50% have an inhibition zone against *Candida albicans* of 10 mm and include in a "strong" category as an antimicrobial.[10]

So far, there is no research regarding discoloration of heat cure acrylic resin after the immersion of Kasturi leaves (*Mangifera casturi*) extracts for denture cleaner. Thus, a study regarding the effect of immersing the solution of Kasturi leaves (*Mangifera casturi*) extracts at a concentration of 50% on the discoloration of heat-cured acrylic resin is required.

MATERIAL AND METHODS

This study was conducted in Biochemistry Laboratory, Universitas Lambung Mangkurat after obtaining a research permit and ethical feasibility No.015/KEPKG-FKULM/EC/II/2021 issued by the ethics committee of the Faculty of Dentistry Universitas Lambung Mangkurat.

This study was a true experimental using pretest-posttest with control group design. The samples of the study used cylindrical heat-cured acrylic resin with a diameter of 15m and thickness of 2mm based on the specifications of ADA No.17 with a smooth, flat and non-porous surface. Samples were separated into 3 groups, immersion group in Kasture leaves extract 50%, alkaline peroxide, and distilled water. Samples were immersed for 5 days based on the effectiveness of alkaline peroxide for 20 minutes in 1 year of use.

Making Heat Cured Acrylic Resin Samples

PMaking acrylic resin samples based on the specifications of ADA No.17 with a diameter of 15mm and thickness of 2mm in a cylindrical shape was conducted in Arsynonsne Dental Laboratory, Surabaya. The first step was applying vaseline over the entire cuvette surface. The top of the cuvette was filled with gypsum plaster type III until it is parallel to the cuvette height. Acrylic mold was put on the top of the gypsum plaster that has been set, and the bottom cuvette was refilled with gypsum plaster type III until fully filled. Acrylic mold was removed after setting gypsum plaster, then the surface of gypsum plaster was smeared with CMS.

The powder and liquid of heat-cured acrylic resin were stirred using the stellon pot with a ratio of 3:1 until the dough stage. The dough was put into the mold that has been smeared with CMS. The cuvette was coated with plastic cellophane, then covered and pressed with a hydraulic press of 1000psi. The cuvette was opened, then plastic cellophane was removed. The excess acrylic resin was cleaned, then pressed again with 2200psi. The cuvette was performed final press using a manual press, then polymerized by boiling in water at 100°C for 30 minutes. Samples that have been polymerized were then taken from the cuvette. After that, the undercut was removed from the surface using the bur frasser and smoother using a bur stone, which was followed using carbide paper with a size of No. 800, 1000, and 2000 until the sample surface was even and smooth.

Making Kasturi Leaves Extracts 50%

Kasturi leaves were chosen according to the criteria, including green, not torn, and leaves located on the third and fourth row of the shoots. 1kg of leaves was cut into small pieces and dried in an oven at 40˚C for 4 hours. Dried Kasturi leaves were crushed using a blender until the simplicia powder was formed and obtained 450 g. 150 gr simplicia powder used was immersed with ethanol 96% in a closed container for 3x24 hours and stirred occasionally. The filtering process was performed 3 times until clear. Extracts were put into the rotary evaporated until they became the concentrated Kasturi leaves extracts. Extracts were evaporated in the water using a water bath at 40˚C until 9 gr thick extract was obtained. Extracts were examined using acetic acid and concentrated sulfuric acid. Kasturi leaves extracts that are free from ethanol solvent did not show any ester odor of ethanol. 200 ml Kasturi leaves extracts at a concentration of 50% were made by mixing 50 ml Kasturi leaves extracts at a concentration of 100% with 150 ml distilled water.

Immersion and Discoloration Measurement

Samples were incubated in saline solution at 37ºC for 24 hours before treatment then dried. Plastic containers were prepared to soak 3 samples according to the number of groups, group A (Kasturi leaves extracts), group B (alkaline peroxide), and group C (distilled water). Every 200 ml of solution was put into each container, and then samples were placed according to the number of samples calculated (8 samples). Samples were immersed for 5 days. This was based on the effectiveness of alkaline peroxide to Candida albicans in 20 minutes of immersion for 1 year of use.

Color measurement was performed using digital analysis with the CIELab system. Digital analysis is a series of tools consisting of the Microsoft LifeCam studio camera with the addition of MATLAB application. The measurement was carried out before and after immersion on Kasturi leaves extracts at a concentration of 50%, alkaline peroxide, and distilled water. Acrylic samples were put into a dark box for shooting using the Microsoft LifeCam studio camera. The distance between the camera lens and samples was 40 cm, and acrylic samples were placed in the middle while adjusting. After the samples were in the appropriate position, the image was taken and saved in JPG format. The discoloration was detected via MATLAB application using the CIELab color system standard recommended by ADA. The color that changed in each sample was calculated with the equation:

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

Notes:

L : Brightness coordinates (Lightness)
a : Red-green chromatic coordinates
b : Yellow-blue chromatic coordinates
L_t, a_t, b_t : Before immersion
L_o, a_o, b_o : After immersion
ΔL : $L_o - L_t$
Δa : $a_o - a_t$
Δb : $b_o - b_t$

RESULTS

The results for the discoloration of acrylic resin immersed in Kasturi leaves extracts 50%, alkaline peroxide, and distilled water measured using digital analysis can be seen in Table 1.

Table 1. The Mean and Standard Deviation in the Discoloration Value of Heat Cured Acrylic Resin

Treatment Group	Mean ± Standard Deviation
Kasturi Leaf Extract 50%	6,75± 1,31
Alkaline peroxide	3,29± 0,57
Distilled water	2,04± 0,60

Table 1 shows that the highest discoloration was in the immersion in Kasturi leaves extracts 50%, while the lowest was in the immersion with distilled water. Data were inserted in the table and processed using SPSS software v.23. The results of the normality test with Shapiro-Wilk Test were obtained a p-value = 0.607 on Kasturi leaves extracts 50% group, p-value = 0.881 on alkaline peroxide group, and p-value = 0.333 on distilled water group. The data showed a p-value >0.05, which means that the data were normally distributed. Then it was continued with the data homogeneity test with Levene's Test. The results were obtained a p-value = 0.167 (p>0.05), which means that data variance was homogeneous. From the results of the tests, data were normally distributed and homogeneous so that parametric analysis using the One Way ANOVA test can be conducted.

The results of the One Way ANOVA statistical test showed p-value = 0.000* (p<0.05), which means that there was a significant difference in treatment groups. In order to find out which groups have a significant difference, a further test must be conducted using Post Hoc Bonferroni. The significance value of the discoloration test of heat-cured acrylic resin can be seen in Table 2.

Table 2. Significance Value of Post Hoc Bonferroni Test for the Discoloration of Heat Cured Acrylic Resin.

	Kasturi Leaf Extract 50%	Alkalin Peroksida	Distilled water
Kasturi Leaf Extract 50%		0,00*	0,00*
Alkaline peroxide			0.033*
Distilled water			

Notes:
* = there is a significant difference (p<0.05)

The results of the Post Hoc Bonferroni test showed that there was a difference in the comparison of each group because of p-value<0.05.

DISCUSSION

Based on the results of discoloration in the heat-cured acrylic resin groups immersed in Kasturi leaves extracts solution at a concentration of 50%, the average sample experienced an increase in L value (3.75), b value (3.50), and a decrease in a value (-3.12). The results of discoloration showed that heat-cured acrylic resin immersion in the Kasturi leaves extracts at a concentration of 50% became lighter, greenish, and yellowish. B-value = 3.5- experienced an increase in the intensity of yellow color. This is because Kasturi leaves extracts containing tannins. According to the study by Gupta, 2016, tannin was found as a yellowish pigment.[11] Tannins have a strong polar structure, resulting in chemical bonds, and are oxygenated in oxygen in the air or water environment. Therefore, discoloration can occur. The discoloration is also influenced by the immersion of acrylic resin for 5 days in the extract solution.[12]

Acrylic resin samples, when immersed in the Kasturi leaves extracts 50%, also experienced a

decrease of a-value = -3.12 so that the intensity of color became green. The discoloration was assumed due to the content of flavonoids and chlorophyll in the extracts. A leaf contains flavonoids that produce yellowish-green color. A leaf also contains chlorophyll with green pigment.[13,14] Change color also occurred in L value = 3.75, which experienced an increase so that the color was lighter. The increase of color occurred due to the preparation of Kasturi leaves extracts using distilled water as diluent. The absorption of distilled water by acrylic resin occurs by diffusion, causing the macromolecules to separate so that it affects the physical properties, resulting in a change color to be lighter.[15]

Phenol compounds have acidic properties that can interfere with the acrylic resin polymeric chain, resulting in the expansion of the polymer bond chains and weakening the physical properties of the resin. Phenol compounds penetrate the acrylic resin surface and interfere with the physical properties, one of which is the discoloration. Phenol releases H^+ ions so that it is possible to bind with CH_3O^-, which is released from the ester group while the benzene group will bind to the RCO group of ester, so the extension of acrylic resin chain bond occurs.[15,16]

Kasturi leaves extracts contain 9.27% flavonoids and tannins. Flavonoids are phenolic derivatives with a basic skeleton of 15 carbon atoms. Two benzene rings (C6) are connected by a propane chain (C3). Unlike flavonoids, tannins are one of the polyphenol compounds. The structure of the tannin compound consists of a benzene ring (C6) bonded to a hydroxyl group (-OH). The chemical chain bonds of tannins have more H^+ than flavonoids. Based on the study by Rifdayanti, 2019, the more acidic and the more hydrogen ions (H^+), the lower the physical properties of heat-cured acrylic resin. This shows that tannins have a great effect on the discoloration of heat-cured acrylic resin immersed in Kasturi leaves extracts 50%.[17,18]

The results of this study are not in line with the study by Zulkarnain, 2017. A study by Zulkarnain examined the discoloration of heat cure acrylic resin immersed in roselle extracts 40%. The results of the study by Zulkarnain show that acrylic resin experienced a significant discoloration after being immersed for 5 days, with a value of $\Delta E \leq 3.3$, while the discoloration value of acrylic resin immersed in Kasturi leaves extracts 50% was $\Delta E \geq 3.3$. Based on the capacity of the human eye to distinguish colors, 3 intervals in assessing discoloration are developed with the ΔE parameter. If discoloration is not visible to human eyes, the value is $\Delta E < 1$. If the discoloration can be accepted clinically, the value is $1 < \Delta E < 3.7$. Meanwhile, if the discoloration is clearly visible and can be accepted clinically, the value is $\Delta E > 3.7$. Discoloration value of Kasturi leaves extracts 50% is $\Delta E \geq 3.3$, which means that discoloration can be clearly visible and cannot be accepted clinically, while discoloration value of immersion in roselle extracts 40% is $\Delta E < 3.3$, which means that it can be accepted clinically. These differences are assumed because roselle extracts contain anthocyanin, which is the red pigments. Red pigment is similar to the base color of acrylic resin.[19,20]

Acrylic resin immersed in the denture cleanser of alkaline peroxide experiences an increase of L value (1.75), a (0.62), and b (0.75). These results show that L value = 1.75 indicates a whiter discoloration. Discoloration on acrylic resin group immersed in alkaline peroxide is caused by the content of sodium perborate. Sodium perborate, when dissolved in water, will form alkaline peroxide. Alkaline peroxide, when formed, results in nascent oxygen [H_2O_2 (hydrogen peroxide) + alkali $\rightarrow$ 2 H_2O_2 + 2O (nascent oxygen)]. Nascent oxygen has a chemical cleaning effect. However, there are side effects related to strong oxidizing agents. The released oxygen causes the oxidation of tertiary amine accelerator or double bonds, which do not react in the acrylic resin matrix, so that it causes the discoloration.[12] This result is in accordance with a study by Chandu, 2015, which stated the use of alkaline peroxide continuously can change color due to its bleaching action.[21]

There is no significant discoloration in the acrylic resin group immersed in distilled water compared to Kasturi leaves extracts 50% group and alkaline peroxide. This is because the content of distilled water is only H_2O (pure water) without other ions. However, the increase of L value (1.25), a value (0.12), and decrease b value (-0.37) occur. The increase of L value and a value is because the acrylic resin has a hydroxyl group (-OH) in its methacrylate compound, while distilled water has a charge of H^+. When -OH is paired with the H^+ group, the diffusion process will occur, resulting in the increase of L value and a value. The decrease of b value is caused by the color absorption of distilled water, which is visually bluish-white. This result is in accordance with the study by Hanifa, 2018, which stated that discoloration occurs on the acrylic resin group immersed in distilled water to be more faded, red, and bluish.[8,22]

The results of the study showed that the acrylic resin group immersed in Kasturi leaves extract 50% had ΔE value = 6.75, alkaline peroxide had ΔE value =3.29, and distilled water had ΔE value =2.04. This shows that acrylic resin immersed in Kasturi leaves extracts 50% experience the greatest discoloration and can be accepted clinically. However, in group immersed in distilled water has the smallest discoloration.[20]

The weakness of this study is the concentrated color of Kasturi leaves extracts at a concentration of 50%. The color of the extract solution has a great influence on the discoloration of heat-cured acrylic resin. The results of the study show that acrylic resin immersed in Kasturi leaves extracts 50% cannot be used as an alternative to natural denture cleanser in terms of color change.

Based on the study above, it can be concluded that there is a significant influence and difference in the immersion of heat cure acrylic resin in kasturi leaves extracts at a concentration of 50% compared to alkaline peroxide and distilled water.

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