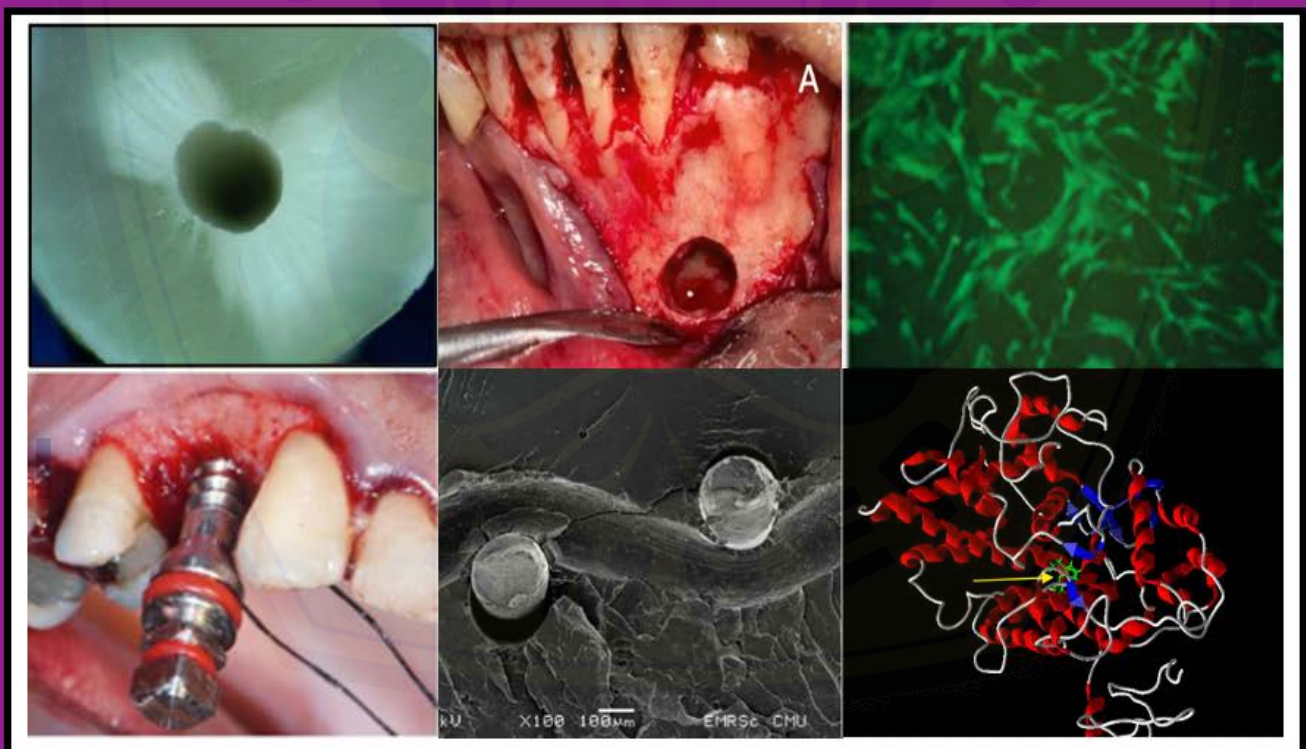


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The Effectiveness of Tobacco Leaf Effervescent Tablets (*Nicotiana Tabacum L.*) 75% against Surface Roughness and Acrylic Color Change

Dewi Kristiana^{1*}, Ady Soesetijo¹, A. Gunadi¹, Rahardyan Parnaadji¹, Amiyatun Naini¹,
Surartono Dwiatmoko², Delvi Fitriani³

1. Department of Prosthodontics, Faculty of Dentistry, Universitas Jember, Jember, Indonesia.

2. Department of Dental Public Health, Faculty of Dentistry, Universitas Jember, Jember, Indonesia.

3. Department of Dental Materials, Faculty of Dentistry, Universitas Brawijaya, Malang, Indonesia.

Abstract

The purpose of this study was to determine the effect of the color stability of acrylic resin dentures and the surface roughness of acrylic resin dentures immersed in denture cleanser agents in the form of effervescent tablets from tobacco leaves (*Nicotiana Tabacum L.*) 75% for 16 days.

The type of this research is a laboratory study with a randomized posttest only control group design. Samples for the surface roughness and color change test were divided into three groups, group 1: the acrylic resin plate was immersed in distilled water (control) for 16 sample days. Group 2: the acrylic resin plate immersed in sodium hypochlorite for 16 days, and lastly, group 3: the acrylic resin plates immersed in tobacco leaf effervescent tablets at 75% concentration for 16 days.. There were significant differences in surface roughness, namely acrylic plates immersed in Aquades, sodium hypochlorite, and 75% tobacco (*Nicotiana tabacum L.*) leaf extract effervescent tablets for 16 days. Acrylic plates immersed in distilled water had a significant difference in color change with acrylic plates immersed in effervescent tobacco leaves (*Nicotiana tabacum L.*) 75%.

On the other side, there was a significant difference in discoloration between the acrylic plates immersed in sodium hypochlorite with the acrylic plate immersed in effervescent tobacco leaf (*Nicotiana tabacum L.*) 75%.

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Introduction

Removable dentures still use acrylic resin as a denture base, because the material is cheap, easy to manipulate and comfortable.¹ Acrylic resin is used as an ideal denture base material, because it has adequate strength, hardness and rigidity, color matches with the color of the tissue that is being replaced, non-toxic, easy to repair and easy to manipulate. The disadvantages of acrylic resin include being able to release residual monomers that can cause allergies, easy to absorb liquids, both water and chemicals, so that they are easily porous, low impact strength so that micro cracks are easy to appear, and are not abrasion resistant.^{2,3}

Denture cleanser is an important action that can be taken to prevent cross-contamination, maintain oral health in particular and the general health of denture and denture wearers will be more durable.⁴ (Souza RF, et al, 2009). Denture stomatitis is a common oral disease among denture wearers. Various etiologic and predisposing factors can cause denture stomatitis. *C. albicans* in the oral cavity can produce hydrolytic enzymes that are toxic, causing Denture Stomatitis.⁵ (Bhat V et al, 2017 8). According to Ribeiro DG et al, 2012) *C. albicans* was most commonly found in dentures (65%), while *S. mutans* was 53.3% and *S. aureus* was 34.4%.⁶ To prevent the occurrence of denture stomatitis, denture wearers must be diligent in cleaning their dentures. Various methods that can be used to clean dentures are mechanical, chemical, or a combination of both. Denture wearers generally prefer to clean their dentures chemically by immersing them in a cleaning solution because it is easier and more effective. To immerse the dentures, they can use

*Corresponding author:

Dewi Kristiana,
Department of Prosthodontics,
Faculty of Dentistry/Universitas Jember, Jember, Indonesia.
E-mail : dewi_kristiana.fkg@unej.ac.id

effervescent tablet denture cleanser agents made from tobacco leaf extract (*Nicotiana Tabacum L.*).

Research on the potential of tobacco leaf effervescent tablets (*Nicotiana tabacum L.*) as denture cleansers to inhibit the growth of *C. Albicans*. The research that has been done by Kristiana et al in 2021 (in press) is that tobacco leaf extracts effervescent tablets (*Nicotiana tabacum L.*) were 75% effective in inhibiting *C. Albicans* with an immersion time of 30 minutes and had the same effectiveness as effervescent tablets on the market. Tobacco leaf extracts effervescent tablets contain tobacco leaf ethanol extract, which is thought to have a chemical cleansing effect on *C. Albicans* due to the active substances and carbon dioxide gas resulting from the reaction of effervescent tablets when dissolved in water. According to Nwachukwu, I. (2018), phytochemical analysis of tobacco leaf extracts showed the presence of carbohydrates, fats and oils, saponins, alkaloids, and tannins.⁷ Tobacco leaves also contain active ingredients, including the phenol group in the form of flavonoids, the alkaloid group in the form of nicotine, the saponin group in the form of steroids, and essential oils in the form of terpenoids.⁸ The active ingredient has antimicrobial activity and is among the tannins; tannic acid has the highest inhibitory activity. Saponins have antifungal properties and are active against *C. Albicans* and other fungi.⁷ Tobacco extract has antifungal activity against *C. Albicans*.⁹ ... Sargassum polycystum seaweed extract, which contains polyphenols, can be used as a denture-cleaning agent because it can inhibit the growth of *S. mutans*, *C. albicans* with a minimum inhibitory value of *Streptococcus mutans* at a concentration of 2.5%, *Candida albicans* at a concentration of 1.25%.¹⁰

Denture cleanser agents should not change either the physical or mechanical properties of the denture base material. However, the use of denture cleaners can actually cause water absorption, causing changes in surface roughness and color stability. Surface roughness promotes adhesion and retention of *C. albicans*, of particular importance in denture-induced stomatitis pathogenesis.¹¹ Denture color stability is an important factor because it affects the aesthetic outcome.¹² Color changes in dentures can be caused by immersion in denture cleanser materials but can also be caused by the habit of

consuming drinks containing dyes.¹³

This study continues the research by Kristiana, et al. (in press) that effervescent tablets of Tobacco leaf extract (*Nicotiana tabacum L.*) are 75% effective in inhibiting *C. albicans* with immersion time of 30 minutes and have the same effectiveness as effervescent tablets on the market. Moreover, the researcher wants to continue looking at the changes in color and surface roughness of acrylic resin dentures soaked in denture cleaning agents effervescent tablets of tobacco leaves (*Nicotiana Tabacum L.*) 75% for 16 days. 16 days is equivalent to wearing dentures for two years with a short time of immersion for 30 minutes a day. This research aims to determine the color stability of acrylic resin dentures immersed in 75% tobacco leaf effervescent tablet (*Nicotiana Tabacum L.*) denture cleanser agent for 16 days and to determine the roughness of acrylic resin denture immersed in tobacco leaf effervescent tablet ((*Nicotiana Tabacum L.*) 75% denture cleanser agent for 16 days.

Materials and methods

The type of research used is experimental laboratory research with the pre-post test control group design. Samples for the surface roughness and color change test were divided into three groups. Group 1: the acrylic resin plate was immersed in distilled water (control) for 16 sample days, and Group 2: the acrylic resin plate was immersed in sodium hypochlorite for 16 days. Group 3: the acrylic resin plates immersed in tobacco leaf effervescent tablets at 75% concentration for 16 days. Acrylic plate measuring 60 X 10 x 2.5 mm for acrylic surface roughness test. The measuring instrument uses a surface roughness tester. The Acrylic plate of 10 X 10 x 2.5 mm for the acrylic color stability test. The measuring instrument uses the Color reader Precise TCR-200.

Tobacco leaf extract is made by drying the tobacco leaves and then extracting it using 70% ethanol. The manufacture of effervescent tablets of tobacco leaf extract is to granulate the tobacco leaf extract that has been obtained with dextrin to produce a granular mass. Then make sour granules by mixing betel leaf granules, citric acid, tartaric acid, and some PVP. Make alkaline granules by mixing sodium bicarbonate with the remaining PVP. Carry out the manufacturing

process at room temperature and maintained air humidity. Add PVP in dry form, then moisten with 70% ethanol drop by drop. Sift the mass to be granulated with a 14 mesh sieve in order to obtain granules with a homogeneous size. Dry the granules in an oven at a temperature of 40-60 °C. Making tablets by flowing a mass of granules into a tablet press machine.

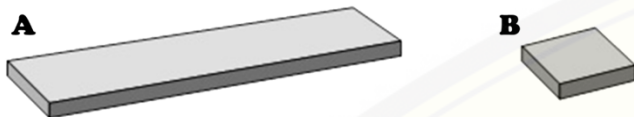


Figure 1. A. 60 X 10 x 2.5 mm acrylic plate for acrylic surface roughness test, B. 10 X 10 x 2.5 mm acrylic plate for acrylic color change test.



Figure 2. A. Tobacco leaf extract effervescent tablet, B. Immersing acrylic plates in various denture cleansers.

Measurements were made after the sample was rinsed with sterile distilled water and then dried using a tissue. The sample is placed on the measuring device on a flat or flat surface, Measurements were made using the Color reader Precise TCR-200 (Figure. 3.A). The measurement result is a value in L, a, b, Comparing the value of the measurement results before and after treatment

In this study, the color change was measured using a color reader and then calculated using the formula:

$$\Delta E = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

Measured using a surface roughness tester (Figure 3. B). Measurements were made after the sample was rinsed with sterile distilled water and then dried. Placing and setting the sample on the measurement site in the form of a smooth and horizontal glass. Each sample is measured on one side only and on 4 different

lines Calculate the average of the three measurement results to get the value of the surface roughness of each plate, Comparing the average value of the measurement results before and after treatment.



Figure 3. A. Acrylic plate color stability test, B. Acrylic plate surface roughness test.

After the research results have been obtained, then tabulate the data according to each group. The normality test of the data used was the Shapiro-Wilk test, and the homogeneity test was carried out using the Levene test, followed by the One-way Anova parametric test with a significance level of 0.05. If significant results are obtained on the One-Way ANOVA parametric test, then it can be tested for multiple comparisons, namely LSD.

Results

The data shows the average color change of acrylic plates immersed in distilled water (control), sodium hypochlorite, tobacco leaf effervescent tablets with a concentration of 75% for 16 days. (ΔE)* and NBS values

Denture cleanser agent	ΔE	SD	NBS Units
Aquadex	0,8115	0.5006	0,7466
Sodium hipoklorit	1,6091	0.9554	1,4804
Tobacco leaf effervescent tablet	3,0993	0.9165	2,8514

Table 1. The average color change of acrylic plates immersed in distilled water (control), sodium hypochlorite, tobacco leaf effervescent tablets with a concentration of 75% for 16 days (ΔE)* and NBS values.

Based on the data obtained from table 1, the average value of the most significant color change on the acrylic plate immersed in 75% tobacco leaf effervescent tablet (ΔE 3.0993) for 16 days, and the lowest average value of the color change on the immersed acrylic plate in distilled water (ΔE 0.8115) for 16 days.

Surface roughness data on samples before and after immersion in each sample group are presented in Table 2.

Group	Average Measurement (µm)		Deviation (µm)
	Before	After	
Aquades	0,1497	1,9296	1,7799
Sodium hypochloric	0,1382	2,4768	2,3386
tobacco leaf effervescent tablet 75%	0,1354	2,6840	2,5486

Table 2. The results of the average surface roughness measurements of acrylic resin samples after immersed in distilled water (control), sodium hypochlorite, 75% concentration of tobacco leaf effervescent tablets for 16 days.

Based on the data obtained from table 2, the most significant average surface roughness value on the acrylic plate was immersed in 75% tobacco leaf effervescent tablet for 16 days, and the lowest average surface roughness value was on the acrylic plate immersed in distilled water for 16 days.

Data analysis in this study begins with the normality test of the data, namely by using the Kolmogorov-Smirnov test. The analysis was continued with the One Way ANOVA test with a 95% confidence level indicating significance .000. Then proceed with the Least Significant Different test to determine the difference between each sample group. Based on the normality test, it was found that the significance value for the acrylic surface roughness test showed a value of .200 ($p > 0.05$), while the acrylic color change test showed a value of .200 ($p > .05$). So it was concluded that the data results of color changes and surface roughness of acrylic resin samples were normally distributed. The homogeneity test results with Levene's test for surface roughness showed a value of .232 ($p > .05$). While the homogeneity for color changes is showing a value of .200 ($p > .05$)

The One Way ANOVA test results for color change and surface roughness both showed a significance value (p) of .000, which means that there was a significant difference, so the LSD (Least Significant Difference) test was then performed to determine which groups had significant differences. The results of the LSD test can be seen in table 3 for color changes and table 4. for surface roughness.

Group	Significance		
	aquades	Sodium hypochlorite	Tobacco leaf (Nicotiana tabacum L.) 75% extract effervescent tablets
Aquades		.112	.000*
Sodium hypochlorite	.112		.006*
tobacco leaf (Nicotiana tabacum L.) 75% extract effervescent tablets	.000*	.006*	

Table 3. Summary of the Least Signification Different (LSD) test of color change on acrylic resin plates after immersion in distilled water, sodium hypochlorite, and effervescent tablets of 75% tobacco (Nicotiana tabacum L.) leaf extract, for 16 days. The * sign indicates a significant value.

Table 3 shows that in the group of acrylic plates immersed in distilled water and acrylic plates immersed in sodium hypochlorite, there was no significant difference in color change (0.112). Acrylic plates immersed in distilled water had a significant difference in color change (.000) compared with acrylic plates immersed in effervescent tobacco leaves (Nicotiana tabacum L.) 75%. The acrylic plates immersed in sodium hypochlorite had a significant color-changing difference (.006) compared with an acrylic plate immersed in effervescent tobacco leaf (Nicotiana tabacum L.) 75%.

Group	Significance		
	aquades	Sodium hypochlorite	tobacco leaf (Nicotiana tabacum L.) 75% extract effervescent tablets
Aquades		.000*	.000*
Sodium hypochlorite	.000*		.000*
Tobacco leaf (Nicotiana tabacum L.) 75% extract effervescent tablets	.000*	.000*	

Table 4. Summary of the results of the Least Signification Different (LSD) test of surface roughness on acrylic resin plates after immersion in distilled water, sodium hypochlorite, and effervescent tablets of 75% tobacco leaf extract (Nicotiana tabacum L.), for 16 days.

Table 4 shows significant differences in surface roughness (.000), namely acrylic plates immersed in Aquades, sodium hypochlorite, and 75% tobacco (Nicotiana tabacum L.) leaf extract effervescent tablets for 16 days.

Discussion

Denture cleanser materials are essential

because they can prevent cross-contamination and maintain patient health, denture longevity, and overall quality of life.¹⁴ This study is an experimental laboratory study to know the effect of effervescent tablets from tobacco leaf extract (*Nicotiana tobacco L*) 75% as denture cleanser against discoloration and surface roughness of acrylic denture base. In this study, samples were tested by measuring the value of color change and surface roughness before and after immersion. A surface roughness tester measured color change using a color reader and surface roughness. Acrylic samples were immersed for 16 days in distilled water, 75% tobacco leaf extract effervescent tablets. Immersing acrylic plates in distilled water, sodium hypochlorite, and effervescent tablets of 75% tobacco leaf extract for 16 days are equivalent to immersing those plates for 30 minutes a day for two years.

Color Change

Denture color stability is an essential factor influencing patient satisfaction so that dentures can be accepted. The color-changing is an indicator of the aging or decay of dental material. Iazzeti.¹⁵ Several factors can contribute to discoloration of dental material after long-term use. These include stain accumulation, water absorption, intrinsic pigment degradation, and surface roughness.

Change can be assessed by colorimetry, which is based on the digital expression of the perceived color of the object. Two color systems used to assess chromatic differences are the Munsell color system and the International Commission de l'Eclairage standard (CIE L*a*b*) color system.¹⁶ The American Dental Association (ADA) recommends using the CIE L*a* system. b*.¹⁷ Color change (ΔE) can be measured from the difference before and after immersion of the acrylic plate in the denture cleanser agent. Color changes are then categorized according to the magnitude of the change value using the National Bureau of Standards (NBS) rating system. NBS unit = $\Delta E \times 0.92$.17 NBS.¹⁸

Description of color difference	NBS unit
Trace	0.0 -0.5
Slight	0.5-1.5
Noticeable	1.5-3.0
Appreciable	3.0-6.0
Much	6.0-12.0
Very much	>12.0

Table 5. Description of color difference according to the NBS.

Table 1 shows the color change (ΔE) of acrylic plates that have been immersed in distilled water, sodium hypochlorite, and 75% tobacco leaf extract effervescent tablets for 16 days which were then categorized according to NBS units. The color change of the acrylic plate, which was immersed in distilled water for 16 days, was .7466. This group experienced the slightest color change, according to the NBS unit, including the slight category. Acrylic plate immersed in sodium hypochlorite for 16 days was 1.4804 according to the NBS unit, including the slight category, acrylic plate immersed in tobacco leaf effervescent tablets for 16 days was 2.8514 according to the NBS unit, including the noticeable category. According to Shamnur (2004), in an in vitro study, the value of color change is clinically acceptable if $E \leq 3.70$.¹⁹ A previous study reported that when the E value was more significant than 3.7, it was the limit of clinical acceptance because the color change was visually detectable.²⁰ Thus, the average acrylic plate immersed in distilled water, sodium hypochlorite, and tobacco leaf extract effervescent tablet was 75% during 16 days is still clinically acceptable. Lai et al. (2003) reported that DE values greater than 2 were considered visually visible, whereas NBS units more significant than 3 were considered unacceptable.²¹

Based on the results of the data analysis, the table above shows that the data is normally distributed, and the results of the homogeneity test show that the data is homogeneous. The third test carried out was the One Way ANOVA test. The One Way ANOVA test results showed that the value was 0.000 ($p < .05$), so it could be seen that there was a difference in the value of the acrylic color change, which was influenced by the type of immersion solution. Denture cleanser by immersion in chemical solutions should not cause any physical, mechanical, or chemical changes in the acrylic resin. The decontamination process can result in surface morphology and changes in flexural strength.²² Effervescent tablets efficiently remove biofilms and stains but can change resin properties.²³ Several studies have investigated the effect of denture cleansers on the physical and mechanical properties of resin dentures. The LSD test table 3 shows that in the group of acrylic plates immersed in distilled water and acrylic plates immersed in sodium hypochlorite,

there was no significant difference in color change (.112). Acrylic plates immersed in distilled water showed a significant difference in color change (.000) with acrylic plates immersed in effervescent tobacco leaves (*Nicotiana tabacum* L.) 75%. Acrylic plates immersed in sodium hypochlorite showed a significant difference in color change (.006) with acrylic plates immersed in tobacco leaf effervescent (*Nicotiana tabacum* L.) 75%. The color change in resin-based dentures could be due to differences in composition and solution where the acrylic resin was immersed. Denture cleaners cause loss of soluble components and plasticizers of resin-based dentures. The higher ionic concentration of denture cleanser than water causes a higher release of dissolved components.¹³

The color change of the denture base resin can also be affected by the type of cleanser. Sodium hypochlorite as a denture cleanser resulted in the whitening of acrylic resin, and many patients were not satisfied with the esthetic results.²⁴ McNeme et al. reported that sodium hypochlorite denture cleanser caused whitening of acrylic resin.²⁵ According to Hong et al., this type of denture cleaner alkaline peroxide causes better color stability of acrylic denture base resin than other cleaners, whereas acid-type cleaners produced the slightest discoloration of the denture base.²⁶ Peracini studied the discoloration of acrylic resin after immersion in various denture cleanser solutions and found no significant difference.²⁷ Phytochemical analysis of tobacco leaf extracts showed the presence of carbohydrates, fats and oils, saponins, alkaloids, and tannins.⁷ Tobacco leaves also contain active ingredients, including the phenol group in the form of flavonoids, the alkaloid group in the form of nicotine, the saponin group in the form of steroids, and essential oils in the form of terpenoids, tannins.⁸ Flavonoids are polyphenolic compounds, acids that can react with polymethylmethacrylate ester compounds in acrylic resins. The acid in contact with the acrylic resin can cause the acrylic resin surface to become weak and easily soluble; this can result in porous on the acrylic resin surface and make it easier for phenol compounds to penetrate the acrylic resin. A hydrolysis reaction occurs and disrupts the acrylic resin polymer chain bonds. This reaction can cause discoloration of the acrylic resin. According to Pinto Lde R, acrylic is easy to

absorb liquid, and it will cause the acrylic to experience microcracks due to repeated absorption. This causes hydrolytic degradation of the polymer by causing damage to the ester linkages and weakening of the polymer bonds.²⁸

Discoloration, surface roughness, halitosis and dimensional changes can be caused by the absorption of the acrylic denture base that is too high. In addition, it can also affect the material properties and result in reduced denture life.²⁹ The denture base can experience discoloration due to intrinsic and extrinsic factors. Acrylic showed the highest average significant colour change after soaking in coffee and ginger stored for 7 (seven) days, followed by PEEK and acetal resin.³⁰ Color changes caused by intrinsic factors occur due to physico-chemical conditions such as variations in temperature and humidity.³¹ Absorption in the oral environment accelerates discoloration and has a more significant effect on materials' chromatic changes than intrinsic factors.³²

Surface roughness

Surface roughness promotes adhesion and retention of *C. Albicans*, thereby causing Denture Stomatitis.³³ Acrylic denture bases have porosity, number of surface free radicals, hydrophobicity, and surface roughness that can affect the attachment of microorganisms to the denture base.³⁴ To avoid retention of *C. Albicans*, the material must have a smooth and polished surface, so that plaque accumulation is minimized or avoided. Based on the results of the data analysis obtained, the data is normally distributed, and the homogeneity test results are homogeneous. Then proceed with the One Way ANOVA test. The results of the One Way ANOVA test showed that the significance value (p) obtained was .000 (< 0.05), so it can be seen that there were differences in the acrylic surface roughness values after being immersed in distilled water, sodium hypochlorite, and 75% tobacco leaf extract effervescent tablets.

Changes in the surface roughness of the denture base are known from the increase in the surface roughness value after treatment compared to before treatment. Table 2 shows that the average surface roughness value increased after the acrylic was immersed in distilled water, sodium hypochlorite, and 75% tobacco leaf extract effervescent tablets for 16 days. Acrylic plates immersed in distilled water, sodium hypochlorite, and 75% tobacco leaf

extract effervescent tablets for 16 days are equivalent to using dentures for two years, each immersing for 30 minutes. The average surface roughness value was measured before and after acrylic immersion in the distilled water group, the average surface roughness value was 0.1497 μm to 1.9296 μm ; this is the lowest average surface roughness value when compared to other groups. The treatment group immersed in 75% tobacco leaf effervescent tablets had an average surface roughness value of 0.1354 μm to 2.6840 μm . The increase in the average surface roughness value of the samples before and after treatment indicated that the roughness of the acrylic plate was increasing.

The clinically acceptable threshold level of surface roughness is 0.2 μm , where no further reduction in plaque accumulation in dentures is expected.³⁵ However, according to Quirynen M and Bollen CM, the surface roughness of less than 2 μm , above will cause colonization of bacteria.³⁶ After the acrylic plate was immersed in distilled water, sodium hypochlorite and 75% effervescent tablets for 16 days experienced an increase in surface roughness which was not within the clinically accepted surface roughness range of 0.2 μm . Acrylic dentures, namely polymethacrylate, are hydrophilic, attracting more water-soluble materials to the surface so that their absorption increases, mainly due to the polar nature of the resin molecules. However, it has been proven that the mechanism is the diffusion of water molecules that penetrate according to the diffusion law, but the bond is still high because the cross-linking agent provides resistance to changes due to denture cleanser agents.¹⁴

Then a one-way ANOVA test was carried out, showing a significant difference (.000.). This significant difference is caused by the active ingredients and the ingredients owned by each cleaning agent. The nature of acrylic that absorbs acrylic has the property of being easy to absorb the surrounding liquid. The acrylic resin polymer chain is diffused and reacts with the acrylic resin polymer chain, causing the acrylic resin polymer bond to break. These molecules can fill the space between polymer chains so that the chemical bond structure can be disturbed and microporosity increases surface roughness.^{37,38} Sodium hypochlorite can damage the interstitial matrix of the acrylic resin, which increases the surface roughness of the acrylic resin.³⁹

The next test that was carried out was the LSD test (table 4.) to find out which groups had significant differences. The results of the LSD test showed that there were significant or significant differences between all groups, namely between groups of acrylic plates immersed in distilled water, sodium hypochlorite, and 75% tobacco leaf effervescent tablets. Acrylic plates immersed in 75% tobacco leaf effervescent tablets experienced a higher roughness value compared to the other groups.

As mentioned before, tobacco leaves contain flavonoids, saponins, alkaloids, and tannins. The active content of tobacco leaves, namely saponins and tannins, are part of phenolic compounds. When the phenolic compounds are in contact with the resin can affect the physical properties of acrylic. Phenol has acidic properties with high polarity, while acrylic resin has low polarity because the acrylic resin is a polymer in the form of long polyester esters consisting of repeating metal methacrylate units. If the ester group reacts with phenol, then the H⁺ ion in the phenol will be released and bind to the CH₃O⁻ which is separated from the ester group, while the benzene group in the phenol will bind to the RCO group of the ester so that it can cause chemical damage and causing roughness of the acrylic resin. Flavonoids are phenolic acidic compounds. The acid contained in 75% tobacco leaf effervescent tablets are thought to cause erosion on the surface of the heat-cured acrylic resin, increasing the surface roughness of the acrylic resin.⁴⁰

Conclusions

There was a difference in color change acrylic plates were immersed in 75% tobacco leaf extract effervescent tablets, distilled water, sodium hypochlorite for 16 days.

There was a difference in color change acrylic plates were immersed in effervescent tablets with 75% distilled water and sodium hypochlorite extract for 16 days.

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Declaration of Interest

The authors report no conflict of interest.

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