World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Potential of tobacco leaf extract paste (*Nicotiana tabacum L.*) as a denture cleanser to prevent denture stomatitis

Amiyatun Naini, Dewi Kristiana *, FX Ady Soesetijo, R Rahardyan Parnaadji, Achmad Gunadi, Afif Surya Adena and Ardhianing Hardita

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

World Journal of Advanced Research and Reviews, 2023, 20(02), 914–921

Publication history: Received on 25 September 2023; revised on 14 November 2023; accepted on 16 November 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.20.2.2313

Abstract

The percentage of use of dentures is relatively high. Dentures with less than optimal cleaning can cause *Candida albicans* infection, called denture stomatitis. The chemical method of cleaning agents from effervescent tablets of tobacco leaf extract has been studied. However, it is still not optimal, so a combination of mechanical cleaning is needed, namely by making a paste preparation. The aim is to analyze the most effective concentration of tobacco leaf extract in acrylic resin denture cleaning paste in reducing the number of *C. Albicans*. Type of laboratory experimental research. There were 4 sample groups, namely A: HPAI herbal toothpaste. B: Tobacco leaf extract paste (PEDT) 25%. C: PEDT 50%. D: 75% PEDT. The number of samples for each group was 10. Making Acrylic Resin Plates size 65 mm × 11 mm × 3.5 mm, Making tobacco leaf extract paste 25%, 50%, 75%. Preparation of *C. albicans* Suspension. Treatment of acrylic plates. Next, calculate the amount of *C. Albicans* using a spectrophotometer. There was a significant difference ($p<0.05$) between the control group, the 25% PEDT group, and the 75% PEDT group. A non-significant difference ($p>0.05$) was found between the control group and the 25% PEDT group and 50 PEDT %, PEDT group 25% to 50% PEDT group, and PEDT group 50% to 75% PEDT group. Effervescent tablets of tobacco leaf extract (*Nicotiana tabacum L.*) are 75% effective in inhibiting *C. Albicans* with 30 minutes of soaking time.

Keywords: Tobacco Leaf Extract Paste; Denture Cleaner; Denture Stomatitis

1. Introduction

Replacing missing teeth in the oral cavity using dentures can restore changes in tissue structure due to the loss of natural teeth, thereby improving mastication, speech, and aesthetic function [1]. The 2018 Riskesdas Indonesia report shows that the incidence of tooth loss among those aged 45-54 years is 0.5%, 55-64 years is 2.6%, and over 65 years is 9%. The percentage of removable denture users aged 45-54 years is 1%, 55-64 years is 2.9%, and over 65 years is 5.8%. Based on this data, it can be seen that the elderly group has experienced a lot of tooth loss and is already using dentures. The consequence that removable denture users often experience if their oral health is poor is denture stomatitis.

Denture stomatitis is an inflammatory reaction in the soft tissue supporting dentures. Fungal infections, especially *Candida albicans*, can cause this condition [2]. According to CADS (Candida-Associated Denture Stomatitis), 60-65% of denture users have experienced Denture Stomatitis with more widespread clinical manifestations, increasing to 75% [3, 4].

Hygienic maintenance of dentures and effectual removal of the microbial film is an essential requirement for the health of denture users. Several mechanical and chemical denture cleaners are available—mechanical methods by brushing teeth with neutral toothpaste or soap. Meanwhile, in the chemical method, the denture is soaked in a product containing

* Corresponding author: Dewi Kristiana

chemicals such as alkaline hypochlorite solution and alkaline peroxide. Previous research stated that tobacco leaf extract effervescent tablets were 75% effective in reducing the growth of *C. albicans* on heat-cured acrylic resin plates due to the activity of bioactive compounds, one of which was polyphenol [5]. Flavonoids, tannins, and saponins inhibit the fungus *C. albicans* [6].

The lack of mechanical cleaning action of chemical methods is a weakness in cleaning dentures soaked in effervescent tablets, so it is still recommended to brush the dentures [7]. Noviyanti [8] supports this in her research, which states that mechanical cleaning of dentures with brushing is more effective for cleaning plaque and does not require a long time. The process of contact with the denture is minimal. At the same time, chemical cleaning requires a long soaking time and, if used continuously, can affect acrylic resin's properties, such as color, hardness, and surface roughness. Therefore, mechanical cleaning is required in addition to soaking.

Brushing with a toothbrush and paste is the most often used mechanical cleaning method because it is considered the most effective in preventing plaque formation. However, achieving a higher level of effectiveness requires a combination of chemical cleaning, which can be obtained from herbal ingredients. Indonesians generally use toothpaste as a denture cleaner because specific denture cleaning paste has yet to be available in Indonesia. Based on the brushing method, respondents mostly clean their removable partial dentures (RPDs) by brushing with a toothbrush and toothpaste. Based on the problems above, this research aims to analyze the most effective concentration of tobacco leaf extract in acrylic resin denture cleaning paste in reducing the number of *C. albicans*.

2. Methods

This research is a laboratory experimental study with a randomized post-test-only control group design. There were four groups of samples, namely Group A: Brush the surface of the heat-cured acrylic resin plate for 30 seconds using an electric toothbrush and HPAI herbal toothpaste. Group B: Brush the surface of the heat-cured acrylic resin plate for 30 seconds using an electric toothbrush and 25% tobacco leaf extract paste (1.2 grams). Group C: Brush the surface of the heat-cured acrylic resin plate for 30 seconds using an electric toothbrush and 50% tobacco leaf extract paste (1.2 grams). Group D: Brush the surface of the heat-cured acrylic resin plate for 30 seconds using an electric toothbrush and 75% tobacco leaf extract paste. (1.2 grams). The number of samples for each group is 10. The sample shape is an acrylic plate with dimensions of 65 mm × 10 mm × 2.5 mm). (American Dental Association Specification No. 12, 1974).

Production of heat-cured acrylic resin plates by preparing a wax model with dimensions of 65 mm × 11 mm × 3.5 mm, then placing it in a cuvette with plaster. Then, remove the wax, stir the heat-cured acrylic resin material using a powder and liquid ratio of 3:1 according to the manufacturer's instructions, and apply the acrylic material. The acrylic resin curing process, finishing, and polishing [9].

Tobacco leaf extract paste is made by drying the tobacco leaves in the oven at 50°C, blending them, and filtering them using a sieve. Macerate tobacco leaves with 96% solvent. The soaking is filtered, and the filtrate is taken. The filtrate is concentrated using a rotary evaporator. Making 25% tobacco leaf paste. Weigh out 25 grams of tobacco leaf extract. Mix 75 grams of placebo ingredients (magnesium carbonate (26%), calcium carbonate (29%), glycerin (6%), propylene glycol (8%), triethanolamine (4%), sterile distilled water (25%), Oleum Menthae Piperithae (2%) then mix homogeneously using a mortar and pastel. Place in a container and close tightly. Making 50% tobacco leaf paste. Weigh 50 grams of tobacco leaf extract. Mix 50 grams of placebo ingredients (magnesium carbonate (26%), calcium carbonate (29%), glycerin (6%), propylene glycol (8%), triethanolamine (4%), sterile distilled water (25%), Oleum Menthae Piperithae (2%). Then, mix homogeneously using a mortar and pastel. Put in container and close tightly. Making 75% tobacco leaf paste and weighing 75 grams of tobacco leaf extract and mixing 25 grams of placebo ingredients (magnesium carbonate (26%), calcium carbonate (29%), glycerin (6%), propylene glycol (8 %), triethanolamine (4%), sterile distilled water (25%), Oleum Menthae Piperithae (2%). Mix homogeneously using a mortar and pastel. Place in a container and cover tightly.

Creation of Sabouraud's Broth Media. Preparation of *C. albicans* suspension. Then, the acrylic resin slab was soaked in sterile saliva for 1 hour and then rinsed with PBS pH 7 (measured with a pH meter) twice for 15 minutes. After contact with saliva, the acrylic plate will immediately be coated with a pellicle, and after 2 hours, plaque will form. The acrylic resin slab was placed in a test tube containing *Candida albicans* suspension and then incubated for 24 hours at 37°C (Figure 1). Next, the acrylic resin slab was inserted and brushed for 30 seconds with four groups of paste (Figure 2). Group A: HPAI herbal toothpaste. Group B: 25% tobacco leaf extract paste (1.2 grams). Group C: 50% tobacco leaf extract paste. (1.2 grams). Group D: 75% tobacco leaf extract paste (1.2 grams), then rinsed with PBS for 15 minutes. The acrylic resin plate was placed in 10 ml of Sabouraud's broth, then vibrated with Virtex in all reaction tubes for 30 seconds to

release *Candida albicans* attached to the acrylic resin plate. Next, count the number of *C. albicans* using a spectrophotometer.

Research data were tabulated according to each group. The normality test used the Shapiro-Wilk test, and the homogeneity test used the Levene test, followed by the parametric One-way ANOVA test with a significance level of 0.05. If the parametric One-Way ANOVA test produces significant results, multiple comparisons can be tested, namely Least Significant Difference (LSD).

Figure 1 Acrylic plate in a test tube containing *Candida albicans* suspension

Figure 2 Brushing acrylic plates

3. Results

The results of research regarding the effectiveness of tobacco leaf extract paste as a heat-cured acrylic resin denture cleaner against *Candida albicans* were carried out by testing the positive control group, namely brushing using herbal toothpaste HPAI (Herba Penawar Alwahida Indonesia) and the treatment group, namely brushing using extract paste leaves with concentrations of 25%, 50%, and 75%, the uptake values of *C. albicans* and Sabouraud Dextrose Broth (SDB) media have been obtained which can be seen in Table 1.

Table 1 Absorbance value of *C. albicans* in Sabouraud Dextrose Broth media

Sample Groups	Control	Brushing with tobacco leaf extract paste		
		25%	50%	75%
1	0.209	0.274	0.180	0.001
2	0.277	0.290	0.163	0.237
3	0.243	0.121	0.195	0.103
4	0.276	0.141	0.125	0.098
5	0.270	0.168	0.152	0.099

The absorbance values obtained in the table above are then converted to obtain the number of *C. albicans* cells on the acrylic resin plate, which is presented in Table 2 with the following formula:

$$N = \frac{(\text{Media absorbance value} + C.albicans) - (\text{Media absorbance value}) \text{ x } X}{\text{The absorbance value of standard solutions Mc. Farland no. 0.5}}$$

N = number of *C. albicans* cells on the acrylic resin plate (CFU/ml)
X = concentration of *C. albicans* in Mc Farland standard solution no. 0.5 = 2×108 CFU/ml

The absorbance value of Sabouraud Dextrose Broth media without mold = 0.254

The absorbance value of standard solution Mc. Farland 0,5 = 0,324

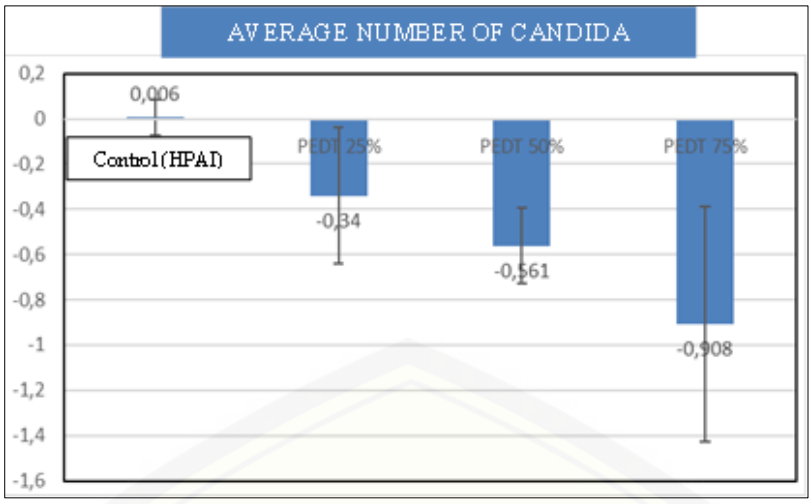
Wavelength used during measurement = 560 nm

Table 2 Number of *C. albicans* cells on acrylic resin after brushing (×108 CFU/ml)

Sample Groups	Control	Brushing with tobacco leaf extract paste		
		25%	50%	75%
1	-0.277	0.123	-0.456	-1.556
2	0.141	0.222	-0.561	-0.104
3	-0.067	-0.820	-0.364	-0.932
4	0.135	-0.697	-0.796	-0.993
5	0.098	-0.530	-0.629	-0.956
Average	0.006	-0.340	-0.561	-0.908
Standard Deviation	0.0803	0.2996	0.1655	0.5187

Based on Table 2, the highest average number of *C. albicans* cells was found in the control group brushing with HPAI herbal toothpaste, namely 0.0006x108 CFU/ml, followed by the 25% concentration tobacco leaf extract paste group, namely -0.340x108 CFU/ml, then the tobacco leaf extract paste group with a concentration of 50%, namely -0.561x108 CFU/ml, then the lowest is the tobacco leaf extract paste group with a concentration of 75%, namely -0.908x108 CFU/ml.

The average results of calculating the number of *C. albicans* cells on acrylic resin plates can be seen as a bar diagram in Figure 3.



Control (+): Brushing with HPAI herbal toothpaste for 30 seconds ; (PEDT) 25%: Brushing with a 25% concentration of tobacco leaf extract paste for 30 seconds; (PEDT) 50%: Brushing with a 50% concentration of tobacco leaf extract paste for 30 seconds ; (PEDT) 75%: Brushing with a 75% concentration of tobacco leaf extract paste for 30 seconds

Figure 3 Diagram of the average number of *C. albicans* cells in each treatment (in 108CFU/ml)

Data analysis in this study was carried out statistically to determine normality, homogeneity, and differences between treatment groups. The research data obtained was tested for normality using the Shapiro-Wilk test and the homogeneity test using the Levene-Statistic test. The normality test is carried out to determine whether the data obtained is usually distributed. The results of the normality test can be seen in Table 3.

Table 3 Normality test results using Shapiro-Wilk

Sample Groups	Sig.
Control	0.270
Brushing tobacco leaf extract paste 25%	0.595
Brushing tobacco leaf extract paste 50%	0.964
Brushing tobacco leaf extract paste 75%	0.366

The results of the Shapiro-Wilk test show a significance or probability (p) value more than 0.05 (p>0.05), so it can be concluded that the data from each group is normally distributed. A data homogeneity test was carried out using the Levene-Statistics test to determine the uniformity of the research sample. The homogeneity test results can be seen in Table 4.

Table 4 Homogeneity test results using the Levene-Statistic test

Levene-Statistic	Sig.
1.309	0.356

The results of the Levene-Statistics test show a significance or probability (p) value of 0.356, which means more than 0.05 (p>0.05), so it can be concluded that the data obtained is homogeneous. Based on the tests that have been carried out previously, it is known that the data is normally distributed and homogeneous.

The analysis was continued with parametric statistical tests using the One Way ANOVA test with a confidence level of 95% (a = 0.05). One Way ANOVA test was carried out to determine whether there were differences between treatment groups. The analysis results using the One Way ANOVA test can be seen in Table 5.

Table 5 One-way ANOVA test results

Data	Sig.
Absorbance	0.012

The One-way ANOVA test results above show a significance value of less than 0.05 (Sig.= 0.012). The test results showed that between each group, there were significant differences in the number of *C. albicans* cells. Differences between groups can be determined using a multiple comparison test, namely the Least Significant Difference (LSD). LSD test results can be seen in Table 6.

Table 6 Least Significance Different (LSD) test results

Groups	Control	PEDT 25%	PEDT 50%	PEDT 7 5 %
Control	-	0.111	0.051	*0.001
PEDT 25%	0.111	-	0.682	*0.046
PEDT 50%	0.051	0.682	-	0.099
PEDT 75%	*0.001	*0.046	0.099	-

*: shows that there are significant differences

Based on the LSD test results in Table 6, it shows that there is a significant difference ($p<0.05$) between the control group and the 25% PEDT group against the 75% PEDT group. There is a non-significant difference ($p>0.05$) was found between the control group against the PEDT 25% and PEDT 50% groups, the PEDT 25% group against the PEDT 50% group, and the PEDT 50% group against the PEDT 75% group.

4. Discussion

The research that has been carried out is a laboratory experimental study that aims to determine the effectiveness of tobacco leaf extract paste (*Nicotiana tabacum L.*) with concentrations of 25%, 50%, and 75% as a heat-cured acrylic resin denture cleaner on the number of *Candida albicans*. *C. albicans* had previously contaminated each research sample and brushed it for 30 seconds. The difference in concentration of tobacco leaf extract paste is to determine the effective concentration as a heat-cured acrylic resin denture cleaner.

Based on the research results, Table 1 shows the average absorbance value of *C. albicans* and its media as measured using a UV-Vis spectrophotometer. The absorbance value is a value that offers the amount of light absorbed by the media solution in each tube to see the density of microbial cells, which will appear as medium turbidity (Optical Density) [10]. The absorbance values obtained were then converted into a formula to get the number of *C. albicans* cells. The results obtained are assumed to be the number of *C. albicans* cells still attached to the surface of the acrylic resin plate after treatment.

Results of the average number of *C. albicans* cells on acrylic resin plates after being soaked in tobacco leaf extract paste (*Nicotiana tabacum L.*) with concentrations of 25%, 50%, and 75%. At a concentration of 75% for soaking the acrylic plate for 30 minutes, the figure was smaller than the concentration of 25%, 50%, and the control. The number of cells of *C. albicans* at a concentration of 75% is smaller, meaning that tobacco leaf extract paste (*Nicotiana tabacum L.*) at a concentration of 75% can inhibit the growth of *C. albicans*. Several cells of *C. albicans* with a concentration of tobacco leaf extract paste (*Nicotiana tabacum L.*) of 75%, smaller than concentrations of 25% and 50%. This is because the higher the concentration of antimicrobial substances, the stronger the antifungal and antibacterial activity.

The results of data analysis using one-way ANOVA also showed significant differences, with the significance value less than 0.05 (Sig. = 0.012). The results show a significant difference between tobacco leaf extract paste (*Nicotiana tabacum L.*) concentrations of 25%, 50%, and 75% and the control group. The significant difference is likely because tobacco leaf extract paste has a more significant effect in inhibiting the number of *C. albicans* colonies than commercially available denture cleaners and sterile distilled water.

Tobacco leaf extract paste contains ethanol extract from tobacco leaves, which is thought to have a chemical cleansing effect on *C. albicans* due to its active substances. Further tests using the LSD test showed that there were significant differences in all groups.

The LSD test results showed that there was a significant difference between the control group and the 25% tobacco leaf extract paste group versus the 75% tobacco leaf extract paste group. A non-significant difference ($p>0.05$) was found between the control group against the 25% tobacco leaf extract paste group and the 50% tobacco leaf extract paste group, 25% tobacco leaf extract paste group against the 50% tobacco leaf extract paste group, and 50% tobacco leaf extract paste group against 75% tobacco leaf extract paste group.

Nwachukwu [11] explained that phytochemical analysis of tobacco leaf extract shows the presence of carbohydrates, fats, oils, saponins, alkaloids, and tannins. Tobacco leaves also contain active ingredients, including phenols such as flavonoids, alkaloids such as nicotine, saponins such as steroids, and essential oils such as terpenoids and nicotine. Alkaloids are the most biologically active components of tobacco [12]. Saponins have antifungal properties and are active against *C. albicans*. Tobacco extract has antifungal activity against *Candida albicans* [13].

Saponin has effective antifungal properties and is active against *Candida albicans* and other fungi. In general, saponins show the ability to complex with sterols in fungal membranes, which can cause loss of membrane integrity. Complex bonds with the lipophilic part of the cell membrane can be formed by saponins, namely sterols, and reduce the surface tension of the membrane and increase permeability so that the biology of the fungus is disturbed [14].

The antifungal mechanism possessed by tannins is due to its ability to inhibit the synthesis of chitin, which forms cell walls in fungi and damages cell membranes to inhibit fungal growth. Antifungal saponins come from the formation of bonds between polar saponin compounds and lipoproteins and adhesives between non-polar saponin groups and fungal plasma membrane fat cells. The mechanism of action of tannin as an antifungal inhibits the biosynthesis of ergosterol, the principal sterol that makes up fungal cell membranes. Sterols are structures and regulatory components found in eukaryotic cell membranes. Sterols are the final product of sterol biosynthesis in fungal cells. Sterols are thought to play a role in fungal cell membrane permeability [14]. This study's cleaning of acrylic resin plates was carried out using mechanical and chemical methods because combining these two methods is quite effective and efficient in killing bacteria and fungi to achieve better cleaning [15]. Mechanical cleaning is achieved through a soft-bristled toothbrush with electrical brushing techniques, and chemical cleaning is achieved through the working mechanism of tobacco leaf extract paste in the cleaning paste. The electric toothbrush used can move horizontally and circularly at a constant speed because it uses electrical energy. The circular movement comes from the brush bristles, which can move in circles, while the horizontal direction is adapted to the frequently used tooth brushing technique. Like brushing natural teeth, the horizontal technique is often used to brush artificial teeth because this method is easy.

Abrasive materials play an essential role in cleaning, but the more complex the abrasive materials contained in toothpaste, the greater the surface roughness produced on acrylic resin. The abrasive ingredients in HPAI herbal toothpaste are calcium carbonate and silica. Based on research by Ramadhan [16], acrylic resin brushed with toothpaste containing silica abrasive and calcium carbonate causes a surface roughness of 0.313µm, where this value exceeds the surface roughness value of dental materials that the oral cavity can accept. Silica has quite hard particles and is relatively large, namely around 8-10µm, so it will produce scratches and significant mechanical effects, potentially increasing the retention of microorganisms and plaque due to surface roughness.

The decrease in the number of *C. albicans* colonies on the acrylic resin plate was also influenced by using an electric toothbrush, which cleans mechanically through the movement of the bristles in direct contact with the acrylic resin plate. The movement of the bristles will remove and destroy the biofilm accumulated on the denture so that microorganisms such as *C. albicans* cannot attach. The brushing cleaning technique is one method that has been proven effective in removing stains and organic deposits on dentures and is the most common cleaning method used routinely. Brushing has been reported to be more effective than soaking for eliminating biofilm. Another advantage of this cleaning method is that it is easy, relatively cheap, and faster. The abrasive ability of the cleaning paste is also a factor in reducing the number of *C. albicans*. The abrasive material contained in the cleaning paste can function to remove plaque to prevent the attachment of *C. albicans*.

5. Conclusion

Tobacco leaf extract effervescent tablets (*Nicotiana tabacum L.*) are 75% effective in inhibiting *C. albicans* with a soaking time of 30 minutes.

Compliance with ethical standards

Acknowledgments

We express our gratitude to the LPPM Universitas Jember for the 2022 KERIS DIMAS research grant provided, which ensured this study's smooth and timely progress.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

[1] Wahjuni S, Mandanie SA. Making Combination Protheses with Castable Extracoronal Attachments. J Vocat Heal Stud. 2017;1(2):75–81.

[2] Hasan S, Kuldeep. Denture Stomatitis: A Literature Review. J Orofac Heal Sci. 2015;6(2):65.

[3] Salerno C, Pascale M, Contaldo M, Esposito V, Busciolano M, Milillo L, et al. Candida-associated denture stomatitis. Med Oral Patol Oral Cir Bucal. 2011;16(2).

[4] Ribeiro DG, Pavarina AC, Dovigo LN, MacHado AL, Giampaolo ET, Vergani CE. Prevalence of Candida spp. associated with bacteria species on complete dentures. Gerodontology. 2012;29(3):203–8.

[5] Kristiana D , Soesetijo A , Gunadi , Parnaadji R , Naini A. Effervescent tablets of tobacco leaves (*nicotiana tabacum l.*) potential as denture cleansers. Journal of Dentomaxillofacial Science (J Dentomaxillofac Sci) 2022; 7(1): 102-108.

[6] Sari KI, Dewi W, Jasrin TA, Sumarsongko T. Denture Hygiene in the Elderly, A Review of Methods and Materials. J Mater Kedokt Gigi. 2018;7(1):1.

[7] Aldo A, Nur AM, W DD. Effect of Effervescent Pineapple Peel Extract in Inhibiting the Growth of Candida Albicans on Heat Cured Acrylic Resin. J Kedokter Gigi. 2016;10(2):135–41.

[8] Noviyanti AM, Parnaadji R, Soesetijo FA. Effectiveness of Using Robusta Coffee Bean Paste as a Denture Cleaner on Surface Roughness of Heat Cured Acrylic Resin. Pustaka Kesehat. 2018;6(2):339.

[9] Wardojo CV, Teguh PB, Rochyani L. Differences in Surface Roughness of Heat Cured Acrylic Resin After Brushing with 30% and 60% Concentrations of Lemongrass Leaf Extract in Toothpaste. Dent J Kedokt Gigi. 2019;13(1):17–24.

[10] Gloria Rambet L, Waworuntu O, Gunawan PN, Studi Pendidikan Dokter Gigi P. Minimum Inhibitory Concentration Test of Pure Garlic Juice (*Allium sativum*) Against the Growth of *Candida albicans*. PHARMACONJurnal Ilm Farm. 2017;6(1):16–23.

[11] Nwachuku IN, Allison L., Chinakwe CE, Nwadiaro P. Studies on the effects Cymbopogon citratus, Ceiba pentandra and Loranthus bengwelensis extracts on species of dermatophytes. J Am Sci. 2008;4(4):52–63.

[12] Fathiazad F, Delazar A, Amiri R, Sarker SD. Extraction of flavonoids and quantification of rutin from waste tobacco leaves. Iran J Pharm Res. 2006;5(3):223–7.

[13] Activities A, Leaf T, Snuff IG. antimicrobial-activities-of-extracts-of-tobacco-leaf-nicotiana-tabacum-and-its-grounded-snuff-utaba-on-candida-albicans-and-strept-2329-891X-1000300.pdf. 2019;7(2).

[14] Arifin Z, Khotimah S, Rahmayanti S. Antifungal activity of ethyl acetate extract of Bacang mango leaves (*Mangifera foetida L.*) against *Candida albicans* in vitro. J Cerebellum. 2018;4(3):1106–19.

[15] Rahmayani L, Sofya PA. Assessment of the level of cleanliness of acrylic removable partial dentures based on brushing cleaning methods and length of use. ODONTO Dent J. 2016;3(1):1.

[16] Ramadhan IPA, Damiyanti M, Triaminingsih S. Effects of brushing with abrasive dentifrices containing various materials on the surface roughness of acrylic resins. J Phys Conf Ser. 2018;1073(6).