

Immersion Of Acrylic Denture In White Pomegranate (*Punica Granatum L.*) Extract As Denture Cleanser Againsts Roughness And Hardness

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ABSTRACT

Background: Prevention of denture stomatitis caused by *Candida albicans* can be done with immersing acrylic denture in disinfectant made from plant, which developed to overcome side effects of chemicals. The antifungal properties of white pomegranate (*Punica granatum L.*) have been established, but the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of white pomegranate (*Punica granatum L.*) against *Candida albicans* were unknown and its effects on physical and mechanical properties of acrylic denture is not yet known. This study aims to analyse MIC and MBC of white pomegranate (*Punica granatum L.*) against *Candida albicans* and analyse the effect of immersion acrylic denture in white pomegranate (*Punica granatum L.*) extract on the physical and mechanical properties of denture.

Method: This study was true experimental laboratory post test only with control group design. After MIC and MBC of white pomegranate (*Punica granatum L.*) against *Candida albicans* were analysed, twenty four acrylic plates were divided into treatment and control groups, then the surface roughness and hardness of acrylic plates were measured after immersed for 5 and 10 days. Data then analysed with Oneway Anova and PostHoc Tukey HSD, Kruskal Wallis, and Mann Whitney.

Result: MIC and MBC of white pomegranate (*Punica granatum L.*) extract againsts *Candida albicans* were 12,5% and 25%. 12,5% white pomegranate (*Punica granatum L.*) extract had surface roughness and hardness value of acrylic plate that comparable with 0,2% Chlorhexidine gluconate.

Conclusion: 12,5% white pomegranate (*Punica granatum L.*) extract can be used as acrylic denture cleanser based on MIC and MBC against *Candida albicans* and surface roughness and hardness testing.

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BACKGROUND

Candida albicans is the most commonly found on denture surfaces and cause denture stomatitis, with a prevalence of 30%-50% in complete denture users.^{1,2,3} Other *Candida* species found on denture surfaces include *Candida tropicalis* and *Candida glabrata*.^{1,4} Local factors contributing to denture stomatitis include dry mouth, aging of the denture, local trauma caused by ill-fitting dentures, poor denture hygiene, continuous and nighttime denture wear, smoking, a high-carbohydrate diet, acidic salivary pH, and sensitivity to denture materials.^{3,5,6} Denture stomatitis can be prevented by regularly cleaning dentures mechanically using a toothbrush or chemically by soaking them in a disinfectant.^{7,5} Chemical denture soaking has proven to be more effective, but denture cleaning products available on the market are generally made from synthetic materials which are known to have side effects after use. A frequently used and effective denture cleaning agent for denture stomatitis is 0.2% Chlorhexidine gluconate. Soaking dentures in 0.2% Chlorhexidine gluconate can cause discoloration of the dentures, tongue stains, and discomfort. This discomfort can be caused by mucosal irritation, ulceration, and changes in taste.^{8,9,6} Contact between denture cleaning agents and denture bases can also cause changes in the physical and mechanical properties of acrylic resin denture bases. Therefore, researchers are exploring alternative denture cleaners from plant materials.^{7,10,6} One plant material that can be used as an alternative denture cleaner is the white pomegranate (*Punica granatum L.*). The flesh of the white pomegranate (*Punica granatum L.*) and its seeds contain many polyphenolic compounds. The phytochemicals identified in white pomegranates (*Punica granatum L.*) include phenolic compounds, such as flavonoids, phenolic acids (ellagic acid and

gallic acid), and hydrolyzable tannins (punicalin and punicalagin) as the main compounds.^{11,12,13}

Previous study state 25% white pomegranate (*Punica granatum L.*) peel extract has an antifungal effect against *Candida albicans*. The antifungal properties of white pomegranate (*Punica granatum L.*) have been established, but the minimum inhibitory concentration and minimum bactericidal concentration of white pomegranate (*Punica granatum L.*) against *Candida albicans* were unknown. Research on the effect of white pomegranate (*Punica granatum L.*) extract on the physical and mechanical properties of heat-cured acrylic denture bases used as denture cleaners is also unclear.¹⁴ Sitorus (2024) showed that the roughness value of heat-cured acrylic plates after immersion in lemongrass (*Cymbopogon nardus*) leaf extract was 25% lower than after immersion in 0.2% chlorhexidine gluconate. Lemongrass leaf extract is known to contain phenolic compounds.¹⁵ There was no significant difference in the roughness of acrylic plates after being soaked in 25%, 37.5%, and 50% Maui banana extract compared to after being soaked in 0.2% chlorhexidine gluconate. Maui banana extract is known to contain tannin compounds.⁷

Chemical denture cleaning with 0.2% chlorhexidine gluconate is performed by soaking the dentures for 20 minutes daily.^{16,7,17} This can cause the denture cleaning agent to penetrate into the pores of the acrylic resin, inhibiting or killing microorganisms, but it can also affect the physical properties of the dentures. The denture cleaning procedure can be carried out over a long period of time because the durability of dentures is 3 to 10 years.^{18,19}

Based on the description above, this study was conducted in two stages, the first stage to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of white pomegranate (*Punica granatum L.*) extract against

Candida albicans, then the second stage to evaluate the effect of white pomegranate (*Punica granatum L.*) extract on the roughness and hardness of heat-cured acrylic denture based on the concentration obtained in the first stage. The roughness and hardness values of heat-cured acrylic resin were measured after immersion for 5 and 10 days. The 5-day period was calculated based on 20 minutes of immersion daily for 1 year, and the 10-day period was calculated based on 20 minutes of immersion daily for 2 years.^{16,7}

RESEARCH METHOD

This study was true experimental laboratory post test only with control group design. The ethical clearance certificate number 682/HRECC.FODM/VI/2023 in faculty of dental medicine Universitas Airlangga. This study was conducted from April 2023 until October 2024. The determination test and preparation of white pomegranate (*Punica granatum L.*) extract were carried out at the UPT Materia Medica Batu, and also the phytochemical testing. The first stage, study was carried out to determine the MIC and MBC of white pomegranate (*Punica granatum L.*) extract against *Candida albicans* at the research centre faculty of dental medicine Universitas Airlangga. The acrylic plates were made at the dental material laboratory faculty of dental medicine IIK Bhakti Wiyata. The surface roughness and hardness testing of acrylic plates were doing at the material technic laboratory department mechanical engineering Universitas Gadjah Mada.

The white pomegranate (*Punica granatum L.*) collected from Situbondo East Java Indonesia were tested for plant determination and the extraction process of white pomegranate (*Punica granatum L.*) was 500 grams of white pomegranates (*Punica granatum L.*) picked at 5 months old were peeled and separated from the

skin. The white pomegranates (*Punica granatum L.*) were pureed in a blender and extracted using the maceration method. The white pomegranates (*Punica granatum L.*) were placed in a sterile container, 96% ethanol was added, and the sterile container was then stirred. The solution was left for 5 days, protected from light, with repeated stirring. After 5 days, the pulp was squeezed out. The white pomegranate (*Punica granatum L.*) solution was filtered using filter paper and a Buchner funnel using a vacuum method. The rotary evaporator evaporates the solvent from the extract solution to obtain a thick extract (Figure1).²⁰

Penelitian ini merupakan penelitian eksperimental laboratorium post test only dengan desain kelompok kontrol. Sertifikat izin etik bernomor 682/HRECC.FODM/VI/2023 di Fakultas Kedokteran Gigi Universitas Airlangga. Penelitian ini dilakukan dari April 2023 hingga Oktober 2024. Uji determinasi tanaman dan pembuatan ekstrak delima putih (*Punica granatum L.*) dilakukan di UPT Materia Medica Batu, serta pengujian fitokimia. Tahap pertama, penelitian untuk menentukan KHM dan KBM ekstrak delima putih (*Punica granatum L.*) terhadap *Candida albicans* dilakukan di research centre Fakultas Kedokteran Gigi Universitas Airlangga. Plat akrilik dibuat di laboratorium IMTKG Fakultas Kedokteran Gigi IIK Bhakti Wiyata. Pengujian kekasaran permukaan dan kekerasan plat akrilik dilakukan di laboratorium Teknik Material Departemen Teknik Mesin Universitas Gadjah Mada.

Buah delima putih (*Punica granatum L.*) yang dikumpulkan dari Situbondo, Jawa Timur, Indonesia, diuji determinasi tanaman dan proses ekstraksi dilakukan dengan mengambil 500 gram buah delima putih (*Punica granatum L.*) yang dipetik pada umur 5 bulan, dikupas dan dipisahkan dari kulitnya. Buah delima putih (*Punica granatum*

L.) dihaluskan dalam blender dan diekstrak menggunakan metode maserasi, ditempatkan dalam wadah steril, ditambahkan etanol 96%, dan wadah steril kemudian diaduk. Larutan dibiarkan selama 5 hari, terlindungi dari cahaya, dengan pengadukan berulang. Setelah 5 hari, ampasnya diperas. Larutan delima putih (*Punica granatum L.*) disaring menggunakan kertas saring dan corong Buchner dengan metode vakum. Evaporator putar menguapkan pelarut dari larutan ekstrak untuk mendapatkan ekstrak kental (Gambar 1).²⁰

Uji fitokimia delima putih (*Punica granatum L.*) berupa uji fenol dan tanin dilakukan dengan menambahkan 3 tetes larutan ferri klorida (FeCl_3) 10% ke dalam 1 ml ekstrak. Hasil positif untuk fenol dan tanin ditunjukkan dengan terbentuknya warna biru tua atau hitam. Uji total fenol dibagi menjadi empat langkah, yaitu pembuatan pereaksi Folin-Ciocalteu 7,5%, pembuatan pereaksi NaOH 1%, pembuatan deret standar, dan pengujian sampel. Persiapan reagen/ pereaksi Folin-Ciocalteu 7,5% dengan cara 7,5 ml reagen Folin-Ciocalteu diambil dengan dipipet dan dilarutkan dengan akuades ke dalam tabung reaksi, hingga volume 100ml, lalu dihomogenkan. Pembuatan reagen NaOH 1% dengan cara 1g NaOH ditimbang dan dilarutkan dalam akuades, hingga volume 100 mL, dan kemudian dihomogenkan. pembuatan deret standar dengan membuat larutan induk asam galat dengan konsentrasi 500 ppm, ditimbang sebanyak 12,5 mg dan dilarutkan dalam metanol dalam labu ukur 25 mL. Larutan stok ini kemudian dibuat menjadi larutan dengan konsentrasi 0, 10, 30, 50, 70, dan 100 ppm dalam labu ukur 25 mL. 1 mL larutan asam galat dipipet ke dalam tabung reaksi, ditambahkan 5 mL reagen Folin-Ciocalteu 7,5%, di-vortex, dan diinkubasi dalam gelap selama 8 menit. Sebanyak 4 mL NaOH 1% ditambahkan ke dalam larutan, di-vortex, dan diinkubasi dalam gelap selama 1 jam. Pengukuran absorbansi dilakukan

menggunakan spektrofotometer pada panjang gelombang 730 nm.^{21,22,23}

Phytochemical test of white pomegranate (*Punica granatum L.*) was the phenol and tannin test was performed by adding 3 drops of 10% ferric chloride (FeCl_3) to 1 ml of extract. A positive result for phenol and tannin was indicated by the formation of a dark blue or black color. The total phenol test was divided into four steps: preparation of 7.5% Folin-Ciocalteu reagent, preparation of 1% NaOH reagent, preparation of the standard series, and sample testing. Preparation of 7.5% Folin-Ciocalteu reagent involves pipetting 7.5 ml of Folin-Ciocalteu and dissolving it with distilled water into a volumetric flask, dissolving it to 100 mL, and then homogenizing it. Preparation of 1% NaOH reagent involves weighing 1 g of NaOH, dissolving it in distilled water, sonicating it to dissolve it, dissolving it to 100 mL, and then homogenizing it. The standard series was prepared by preparing a stock solution of gallic acid with a concentration of 500 ppm. 12.5 mg was weighed and dissolved in methanol in a 25 mL volumetric flask. This stock solution was then prepared into solutions with concentrations of 0, 10, 30, 50, 70, and 100 ppm in 25 mL volumetric flasks. 1 mL of the gallic acid solution was pipetted into a test tube, 5 mL of 7.5% Folin-Ciocalteu reagent was added, vortexed, and incubated in the dark for 8 minutes. 4 mL of 1% NaOH was added to the solution, vortexed, and incubated in the dark for 1 hour. Absorbance measurements were performed using a spectrophotometer at a wavelength of 730 nm.^{21,22,23}

To determine the MIC and MBC of white pomegranate (*Punica granatum L.*) extract against *Candida albicans*, *Candida albicans* was grown using sterile tubes in liquid media and incubated for 48 hours anaerobically at 37°C. Turbidity was observed and standardized with a 0.5 McFarland

standard. Several test tubes were prepared for the serial dilution technique. The first test tube was filled with 10ml of 100% extract. The second and subsequent test tubes were filled with 5ml of BHIB medium each. 5ml of the extract from the first test tube was taken and transferred to the second test tube and homogenized. 5ml of the second test tube was taken and transferred to the third test tube and homogenized. This process was repeated with the subsequent test tubes, and so on, until 5ml was taken from the last test tube. 0.1ml of the bacteria was added to each dilution tube and incubated for 48 hours anaerobically at 37°C. Then, the turbidity in each dilution tube was observed. Then, 0.1 ml was taken from each dilution tube and planted on saboraaud dextrose agar media using the spreading technique and incubated for 48 hours anaerobically at 37°C. Afterward, the number of colonies growing on the agar media was observed and counted using a colony counter with colony forming units CFU/ml (Figure2).

The research samples were determined using the Frederer formula, $t(r - 1) > 15$. t was the number of treatment groups, r was the number of samples per treatment group. In this study, there were 8 groups.²⁴ Group 1 was acrylic plates soaked in MIC white pomegranate extract for 5 days. Group 2 was acrylic plates soaked in MIC white pomegranate extract for 10 days. Group 3 was acrylic plates soaked in MBC white pomegranate extract for 5 days. Group 4 was acrylic plates soaked in MBC white pomegranate extract for 10 days. Group 5 was negative control group, acrylic plates soaked in distilled water for 5 days. Group 6 was negative control group, acrylic plates soaked in distilled water for 10 days. Group 7 was positive control group, acrylic plates soaked in 0.2% chlorhexidine gluconate for 5 days. Group 8 was positive control group, acrylic plates soaked in 0.2% chlorhexidine gluconate for 10 days. Based on the

results obtained through the calculation above, the r value ≥ 3 so the number of samples required is 3. The total number of samples used is 8×3 samples = 24 samples.

The pH measurement of white pomegranate (*Punica granatum L.*) extract was carried out by inserting litmus paper into the white pomegranate (*Punica granatum L.*) extract that had been diluted according to the MIC and MBC, then removed and adjusted to the color code of the litmus paper. Next, the pH measurement of 0.2% chlorhexidine gluconate and distilled water was carried out.

Heat-cured acrylic plate sample preparation steps were begun by creating a mold space using red wax, shaped according to ISO standards, a 65x10x3mm rectangle. Vaseline was applied to the top and bottom cuvettes with a brush in a thin, even layer. Type III plaster mix was prepared by mixing water and plaster until homogeneous, then pouring it into the bottom cuvette until the mixture reaches the edge of the cuvette. The red wax was applied and gently pressed until the surface was partially embedded. The plaster was allowed to set, then vaseline was applied. The top and bottom cuvettes were combined, then the Type III plaster mix was poured until full and allowed to set. The red wax was removed by immersing the cuvette in boiling water. Remove the cuvette, open it, and apply a sufficient amount of CMS to the plaster surface using a brush. Acrylic plate preparation involves mixing the polymer and monomer in a 3:1 ratio in a sterile pot, then stirring until homogeneous, reaching the dough stage. The mixture was poured into the mold, then a piece of cellophane was placed on top, and the top of the cuvette was covered. Pressing was performed using a hydraulic press at 1000 psi (70 kg/cm²). The cuvette was opened, the excess acrylic was cut off, and the cuvette was closed again. A final press was performed at 2200 psi (154 kg/cm²). The cuvette

was boiled in boiling water for 30 minutes, then allowed to cool in room temperature (37°C), and the sample was removed from the cuvette. The acrylic resin sample was removed from the cuvette and a total of 24 samples were prepared. Sharp and rough areas of the sample were smoothed using a frazer bur. The surface was smoothed using a rotary grinding machine and sandpaper. Polishing was carried out sequentially with grits 800, 1000, 1500, and 2000 until the sample surface was smooth. The final polishing stage was using pumice to achieve a shiny surface.²⁵

Acrylic plate roughness measurement tool was a profilometer surface roughness tester. The sample was placed on a metal block and the plate was fixed. The height of the profilometer stylus was adjusted to match the surface of the acrylic plate. The surface of the plate being measured for roughness was marked using a marker.¹⁶ Acrylic plate hardness measurement tool was a Vickers hardness tester. The indentation process was performed at least three times on each sample at different locations, and then the average diagonal was taken.²⁶

The data were analyzed using IBM SPSS Statistics. Data normality was tested using the Shapiro-Wilk because the total samples in this study was less than 50. If the data were normal, a homogeneity test was performed. If the data were homogeneous, can be continue with the significance test OneWay ANOVA and if there was a significant difference in the sample group, to determine which groups exhibited significant differences, post-hoc Tuckey's HSD was used. But if the data were not normal, a transformation test should be done. If the data were still not normal, can be continue with the significance test Kruskal Wallis and if there was a significant difference in the sample group, to determine which groups exhibited significant differences, Mann Whitney was used.²⁷

RESULTS

The results of the roughness test on acrylic plates show roughness results in units of μm .²⁸ The results of the acrylic plate hardness test show the hardness results in VHN units.²⁶



Figure 1. The white pomegranate (*Punica granatum* L.) (a) The white pomegranate (*Punica granatum* L.) powder (b) The white pomegranate (*Punica granatum* L.) extract (c).

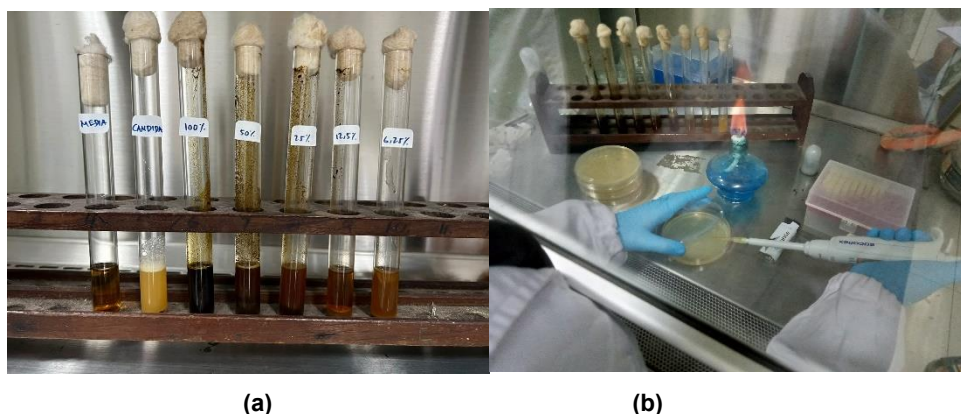
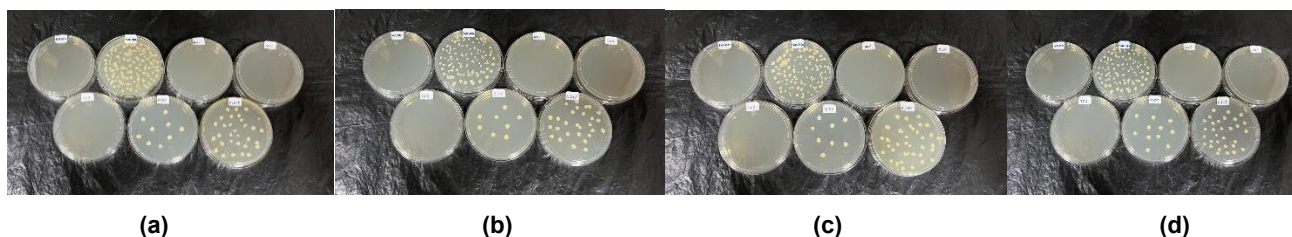


Figure 2. The serial dilution technique (a) The spreading technique (b).

Larutan Blanko	Tanin / Fenol	No	Parameter Uji	Metode	Hasil	Keterangan
		1.	Tanin / Fenol	Pewarnaan dan Pengendapan Harborne, J.B., 1996	(+) Positif	
		2.	Total Tanin	Spektrofotometri UV-Vis FHI Jilid II, 2017	15,4020 mg ETA / g	

(a) (b)

Figure 3. A positive result colour for phenol and tannin was indicated by the formation of a dark black color (a) the phytochemical of phenol and tannin quantitative test (b).



(a) (b) (c) (d)

Figure 4. MIC and MBC of white pomegranate (*Punica granatum L.*) extract against *Candida albicans* sample number 1 (a) sample number 2 (b) sample number 3 (c) sample number 4 (d).



(a) (b) (c) (d)

Figure 5. pH measurement of MIC white pomegranate (*Punica granatum L.*) extract (a) MBC white pomegranate (*Punica granatum L.*) extract (b) Chlorhexidine gluconate 0,2% (c) distilled water (d)

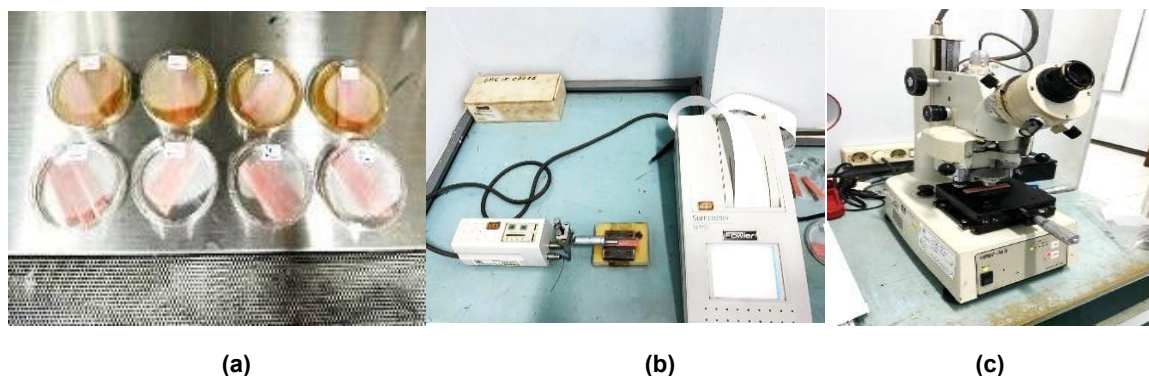


Figure 6. Immersing acrylic plates samples in treatment and control group for 5 days and 10 days (a) Surface Roughness Tester (b) Vicker Hardness Tester (c).

Table 1. MIC and MBC of white pomegranate (*Punica granatum L.*) extract against *Candida albicans*

No.	Control Media	Control Candida	100%	50%	25%	12,5%	6,25%
1	-	160	-	-	-	9	33
2	-	151	-	-	-	8	36
3	-	154	-	-	-	8	30
4	-	145	-	-	-	11	31

* CFU/ml

Table 2. Acrylic plate roughness test results

Group	Mean $\pm$ SD (μm)
Extract 12,5% 5 days	2.84 $\pm$ 0.17
Extract 12,5% 10 days	3.53 $\pm$ 0.24
Extract 25% 5 days	3.62 $\pm$ 0.10
Extract 25% 10 days	3.92 $\pm$ 0.04
Distilled water 5 days	2.08 $\pm$ 0.05
Distilled water 10 days	2.30 $\pm$ 0.34
Chlorhexidine 0,2% 5 days	2.64 $\pm$ 1.20
Chlorhexidine 0,2% 10 days	3.33 $\pm$ 1.39

Table 3. Acrylic plate hardness test results

Group	Mean $\pm$ SD (VHN)
Extract 12,5% 5 days	16 $\pm$ 1
Extract 12,5% 10 days	15.33 $\pm$ 1.15
Extract 25% 5 days	14.33 $\pm$ 0.57
Extract 25% 10 days	13.33 $\pm$ 0.57
Distilled water 5 days	18 $\pm$ 1
Distilled water 10 days	15.67 $\pm$ 0.57
Chlorhexidine 0,2% 5 days	17 $\pm$ 2.646
Chlorhexidine 0,2% 10 days	15 $\pm$ 0.00

Table 4. Normality test Saphiro Wilk of data acrylic plate roughness

Group	Sig.
Extract 12,5% 5 days	0.843
Extract 12,5% 10 days	0.888

Extract 25% 5 days	0.702
Extract 25% 10 days	0.463
Distilled water 5 days	0.747
Distilled water 10 days	0.606
Chlorhexidine 0,2% 5 days	0.771
Chlorhexidine 0,2% 10 days	0.275

Table 5. Homogeneity test Levene test of data acrylic plate roughness

Levene statistic	df 1	df 2	p-value
1,400	7	16	0,271

Table 6. Significance test OneWay Anova of data acrylic plate roughness

	Sum of Squares	df	Mean Square	F	Sig.
Antar kelompok	9.280	7	1.326	2,915	0,036
Dalam kelompok	277	16	0,455		
Total	16.557	23			

Table 7. Hasil analisis post-hoc Tuckey's HSD of data acrylic plate roughness

Group	Extract 25% 5d	Distilled water 5d	Chlorhexidine 5d	Extract 12.5% 10d	Extract 25% 10d	Distilled water 10d	Chlorhexidine 10d
Extract 12.5% 5d							
Extract 25% 5d	0.172	0.190	0.726	0.228	0.067	0.344	0.384
Distilled water 5d		0.013*	0.093	0.863	0.602	0.029*	0.602
Chlorhexidine 5d			0.327	0.019*	0.004*	0.699	0.038*
Extract 12.5% 10d				0.127	0.034*	0.546	0.228
Extract 25% 10d					0.489	0.041*	0.726
Distilled water 10d						0.010*	0.303
							0.080

Table 8. Normality test Saphiro Wilk of data acrylic plate hardness

Group	Sig.
Extract 12,5% 5 days	1,000
Extract 12,5% 10 days	<,001
Extract 25% 5 days	<,001
Extract 25% 10 days	<,001
Distilled water 5 days	1,000

Distilled water 10 days	<,001
Chlorhexidine 0,2% 5 days	,363
Chlorhexidine 0,2% 10 days	.

Table 9. Normality transformation test of data acrylic plate hardness

Group	Sig.
Extract 12,5% 5 days	.966
Extract 12,5% 10 days	<,001
Extract 25% 5 days	<,001
Extract 25% 10 days	<,001
Distilled water 5 days	.969
Distilled water 10 days	<,001
Chlorhexidine 0,2% 5 days	.318
Chlorhexidine 0,2% 10 days	<,001

Table 10. Kruskal Wallis test of data acrylic plate hardness

Group	n	mean	p-value
Extract 12,5% 5 days	3	15.33	0,036
Extract 12,5% 10 days	3	12.33	
Extract 25% 5 days	3	6.83	
Extract 25% 10 days	3	2.67	
Distilled water 5 days	3	21.50	
Distilled water 10 days	3	14.17	
Chlorhexidine 0,2% 5 days	3	16.67	
Chlorhexidine 0,2% 10 days	3	10.50	

Table 11. Mann Whitney test of data acrylic plate hardness

Group	Extract 25% 5d	Distilled water 5d	Chlorhexidine 5d	Extract 12.5% 10d	Extract 25% 10d	Distilled water 10d	Chlorhexidine 10d
Extract 12.5% 5d	0.072	0.07	0.513	0.487	0.046*	0.063	0.121
Extract 25% 5d		0.046*	0.246	0.239	0.099	0.068	0.114
Distilled water 5d			0.822	.046*	.046*	0.046*	0.037*
Chlorhexidine 5d				0.369	0.072	0.507	0.487
Extract 12.5% 10d					0.068	0.796	0.480
Extract 25% 10d						0.043*	0.034*
Distilled water 10d							0.114

DISCUSSION

Candida albicans was the most common fungus found on denture surfaces and palatal mucosa, causing denture stomatitis, with a prevalence of 30%-50% in complete denture users.^{1,2,3} Chemical denture cleaning, which involves soaking the dentures in plant-derived cleaning agents, has been developed to address the side effects of synthetic materials.^{9,10} The results of the determination of the plant used in this study were indeed white pomegranate (*Punica granatum L.*). The purpose of this determination test was to determine the authenticity of the white pomegranate (*Punica granatum L.*) sample used in this study to avoid errors and mix-ups with other plants.

Phytochemical testing of white pomegranate (*Punica granatum L.*) extract in this study showed that the extract contained phenol and tannin compounds with a total content of 15.4020 mg/EGA/g. The results of the MIC and MBC tests against *Candida albicans* in this study indicated that the white pomegranate (*Punica granatum L.*) extract had antifungal activity against *Candida albicans* with an MIC of 12.5% and a MBC of 25%. This indicates that the higher the concentration of white pomegranate (*Punica granatum L.*) extract, the greater the antifungal activity due to the higher active ingredient content.

White pomegranate (*Punica granatum L.*) extract contains phenol and tannin compounds. Phenolic compounds can inhibit RNA and DNA synthesis of *Candida albicans*. Phenolic compounds also exhibit antifungal activity through inhibition of ergosterol, a component of fungal cell membranes, and glucosamine, which plays a role in the synthesis of chitin, a component of the *Candida albicans* cell wall. Tannin compounds inhibit the growth of fungal microbes by causing an imbalance in *Candida albicans* metabolism by inhibiting

oxidative phosphorylation and iron.²⁸ Tannin compounds, as antifungals, can inhibit the activity of glycosyltransferase enzymes, which play a role in the formation of monosaccharides into polysaccharides, the main component of the *Candida albicans* cell wall. The integrity of polysaccharides will be disrupted if glycosyltransferase enzyme activity was inhibited.²⁹

Measurement of the pH value of each ingredient showed that pH of 12.5% white pomegranate (*Punica granatum L.*) extract was 4, the pH of 25% white pomegranate (*Punica granatum L.*) extract was 3, the pH of distilled water was 7, and the pH of 0.2% chlorhexidine gluconate was 6. Then, the acrylic plate samples were soaked for 5 and 10 days, and the acrylic plate samples were tested for surface roughness and hardness.

The results of this study showed that the roughness value of the acrylic plate group was lower in the 5-day distilled water group compared to the 5-day 25% extract group, the 10-day 12.5% extract group, the 10-day 25% extract group, and the 0.2% chlorhexidine gluconate group. The roughness value of 10-day distilled water group had lower than the 5-day 25% extract, 10-day 12.5% extract, and 10-day 25% extract groups. The roughness value of the 5-day 0.2% chlorhexidine gluconate group had lower than the 10-day 25% extract group.

This is due to the water absorption capacity of acrylic resin. Polymer-based acrylic resins absorb water into their matrix through a controlled (continuous) diffusion process. Water molecules can penetrate the polymethyl methacrylate mass and occupy positions between the polymer chains, causing them to separate. Over time, this process increases the distance between polymers and causes matrix expansion, leading to increased

porosity and increased roughness of the acrylic resin.^{30,31}

Distilled water that contains pure H₂O, unlike mineral water which was a universal solvent that can easily dissolve various particles. Distilled water does not contain active compounds when compared to white pomegranate extract (*Punica granatum L.*) which contains phenol and 0.2% chlorhexidine gluconate which contains chlorine. If the phenol compound contact with acrylic resin, it can cause chemical damage to the resin surface. The phenol compound (C₆H₅OH) has a hydroxyl group (-OH) which is acidic with high polarity, while acrylic resin was a polymer with a long poly ester form consisting of repeating methyl methacrylate with low polarity ((C₅O₂H₈)_n). Likewise, the chlorine contained in chlorhexidine has a chemical damaging effect which causes degradation of ethylene glycol dimethyl methacrylate.^{7,15}

Acidic compounds will release H⁺ ions from their hydroxyl groups and cause the formation of phenoxide anions (C₆H₅O⁻). When the ester group of acrylic resin reacts with phenol, the H⁺ ions in the phenol will be released and bind to CH₃O⁻ which was released from the ester group, while the phenoxide anions (C₆H₅O⁻) in the phenol will bind to the acyl functional group (RCO⁺) of the ester group. This ion exchange reaction causes the degradation of polymer bonds so that some bonds will break free and the incoming solvent molecules will occupy positions between the polymer chains so that the polymer chains separate which causes the chemical bonds of the acrylic resin to become unstable and chemical damage occurred on the surface of the acrylic resin.^{30,31}

The results of this study showed that the hardness value of the acrylic plate in the 25% extract group for 10 days was lower than that in the 12.5% extract group for 5 days, distilled water for 10 days, and 0.2% chlorhexidine gluconate for 10

days. The hardness value of the distilled water group for 5 days was higher than 25% extract group for 5 days, 12.5% extract for 10 days, 25% extract for 10 days, distilled water for 10 days, and 0.2% chlorhexidine gluconate for 10 days. This was because the 25% white pomegranate (*Punica granatum L.*) extract has a higher concentration than the 12.5% extract, that was mean the phenolic compound content was also higher besides the soaking time was 10 days, so the degradation process of the polymethyl methacrylate polymer bond was greater, thus resulting in a decrease in the hardness of the acrylic resin.³² Distilled water does not contain active compounds and the pH of the distilled water was neutral, in addition, the soaking time was 5 days, so that the degradation process of the polymer bonds of polymethyl methacrylate that occur was smaller and produce higher acrylic resin hardness.³³

The results of this study showed that the roughness and hardness values of acrylic plates were insignificant on 12.5% extract group for 5 days with distilled water, and the 12.5% extract group for 5 days and 10 days with 0.2% chlorhexidine gluconate for 5 days and 10 days. Although the roughness and hardness of 12.5% white pomegranate (*Punica granatum L.*) extract with distilled water were insignificant, 12.5% white pomegranate (*Punica granatum L.*) extract had antifungal activity against *Candida albicans*, while distilled water did not. Chlorhexidine gluconate 0.2% and 12.5% white pomegranate (*Punica granatum L.*) extract showed no significant effect on the roughness and hardness of acrylic resin surfaces and had antifungal activity against *Candida albicans*. Therefore, 12.5% white pomegranate (*Punica granatum L.*) extract can be used as an alternative denture cleaning agent.

CONCLUSION

The MIC and MBC of white pomegranate (*Punica granatum L.*) extract against *Candida albicans* were 12.5% and 25%, respectively. The effect of immersion in 12.5% white pomegranate (*Punica granatum L.*) extract on the roughness of acrylic plates was comparable with 0.2% chlorhexidine gluconate. The effect of immersion in 12.5% white pomegranate (*Punica granatum L.*) extract on the hardness of acrylic plates was comparable with 0.2% chlorhexidine gluconate. 12,5% white pomegranate (*Punica granatum L.*) extract can be used as acrylic denture cleanser based on MIC and MBC against *Candida albicans* and surface roughness and hardness testing.

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