DENTINO
JURNAL KEDOKTERAN GIGI
Vol II. No 2. September 2017

THE COMPARISON OF ANTIFUNGAL EFFECT BETWEEN GARLIC EXTRACTS AND ALKALINE PEROXIDE TOWARDS CANDIDA ALBICANS AT ACRYLIC HEAT CURED

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ABSTRACT

Background: *Candida albicans is a microorganism found on denture plaque. Candida albicans can penetration and accumulated on surface of the denture and then to infection the soft tissue that causes denture stomatitis. Denture cleanser has effectivity of anti-fungal is alkaline peroxide. Naturally denture cleanser contain of anti-fungal is garlic.* ***Purpose:*** *This research is to analyze inhibition effectivity of garlic extract concentration* 2,5%, 5%, 10%, 12,5%, 15%, *and alkaline peroxide to Candida albicans growth on acrylic heat cured.* ***Method:*** *This research uses true experimental with post test only with control group design. Garlic extract concentration 2.5%, 5%, 10%, 12.5%, 15%, and alkaline peroxide. The method of this research uses diffusion method with the measure of inhibition zone.* ***Result:*** *The result of the research garlic extract concentration 2.5%, 5%, 10%, 12.5%, 15%, and alkaline peroxide to Candida albicans growth have inhibition zone are 11.29mm, 13.25mm, 16.30mm, 18.2mm, 19.46mm, and 15.33mm. The result of normality test with Saphiro-Wilk is p>0,05, Levene's test that results is p=0,575 (p>0,05) so the all data are normality and homogenity. Analyze data uses One Way ANOVA that result is p=0,000 (p<0,05) are differences inhibition effectivity and next testing of Post Hoc Bonferroni show that significant differences between each group.* ***Conclusion:*** *The research is the differences inhibit effectivity of the garlic extract concentration* 2,5%, 5%, 10%, 12,5%, 15%, *and alkaline peroxide to Candida albicans growth on acrylic heat cured. Garlic extract in 10%, 12.5% and 15% have a bigger effectivity of inhibition than alkaline peroxide to Candida albicans growth.*

Key words: *Garlic extract, Alkaline peroxide, Candida albicans*

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INTRODUCTION

Complete denture, (also known as false teeth), are prosthetic devices constructed to replace missing teeth. Conventional dentures are removable and used to chew, speak and aestethics looks.

Denture consists of denture elements and denture bases. Removable denture bases made of metal materials and acrylic polymethyl methacrylate resin. Acrylic resin consists of monomers in liquid form and polymers in powder form. It has introduced since 1937. Acrylic resin heat cured type has several advantages; good aesthetic value, non-toxic, cheap and easy to process, good biocompatibility towards oral tissues, good color stability, not irritating, and it has no odor and taste. Acrylic resin has also disadvantages ie: dimension instability, water absorption, discoloration, and roughness in a long period of use. Complete denture with acrylic resin heat cured type will be in contact with saliva, food and drink in oral cavity. This causes plaque and stains. Careless in cleaning will lead to rough surface of the denture.

Chlorhexidine is a kind of mouthwash which is used widely and effectively to prevent plaque formation. Chlorhexidine contains positive charges that are guanid and fenol which have antiseptics and disinfecting capabilities with wide spectrum. It is very effective to eradicate the gram-positive bacteria, gram negative bacteria, yeast bacteria, sponge and protozoa. immerse denture in chlorhexidine solution once a day for 20 minutes. The main benefit of chlorhexidine in addition to antiseptics and anti-fungus is its ablility to make a direct chain with the polysaccharide layer that envelops bacteria so that it has difficulty to stick on the surface of teeth.

The ability of Chlorhexidine in making direct chain with its alcohol content causes abration on the surface of acrylic resin. That is why in a long period of use, chlorhexidine makes the surface of acrylic resin to be rough. Rough surface of acrylic resin bases will lead to prosthesis discoloration, microbial colonization, bio-film formation that increases the roughness of acrylic resin surface so the patient is getting uncomportable. There are many researchs done in developing traditional medicines derived from plants to be used as mouth wash functioned as antiseptics and disinfectans, bay leaves for example.

Bay leaves are essential oil-producing plants. It has eugenol and phenol varying percentage from 4.40 to 8.56%. Essential oils are known as anti-fungal and anti-bacterial. Rough surface and disorder of acrylic resin bases are a beginning of the accumulation of deposits on dentures, the increase of discoloration and microorganisms in mouth affect the aesthetics of dentures. Other studies have shown that phenols cause the surface of acrylic resin to be rough. The effect of immersion of acrylic resin bases into 25% bay leaves extract can inhibit candida albicans growth but it also has side effect that is the increasing roughness of acrylic resin bases surface. Phenols which has contact with acrylic resins causes chemical damage to the surface of the acrylic resin bases. The purpose of this study was to analyze the effect of immersion of 25% bay leaves extract on the surface roughness value of heat cured acrylic resin type compared with 0,2% chlorhexidine gluconate solution and aquadest.

MATERIALS AND METHODS

This research is a laboratory experimental research. The research used is post-test only control group design. This research was conducted in the laboratory of Dentistry Faculty of Lambung Mangkurat University for the manufacture of heat cured acrylic bases resin sample. Bay leaves extracts made in biochemistry laboratory of medical faculty of Lambung Mangkurat University Banjarbaru. Sample test of roughness on the surface of heat cured type acrylic resin was carried out in manufacturing process Laboratory of mechanical engineering Technic Department of the Industrial Technology Faculty of Institut Teknologi 10 November.

Materials used in this research are wax model, polymer powder and monomer liquids heat cured type of acrylic resin, cast, cold mould seal, vaseline, cellophane, sandpaper, saline solution, 25% concentration of bay leaves methanol extract, 0,2% Chlorhexidine gluconate and aquadest. Tools used in this research are bowl, spatula, tweezers, knives, cuvette, scales, test tube, measuring cylinder, vibrator, hydraulic bench press, stove, pan, bur stones, straight handpiece, surface roughness tester

The research procedure began with the manufacture of heat cured acrylic type resin sample as much as 42 samples with size of 65 x 10 x 3.3mm refer to ISO 1567 standards. The stages of making heat cured acrylic resin was to prepare red wax that had been cut with size of 65x10x3.3mm. Lower cuvette was filled with dough of casts and was then vibrated, after that, the red wax which had been cut was planted into the cuvette at the same surface with the cast dough. When the casts was being hardened, its surface smeared with vaseline. The upper cuvette was fitted and filled with the casts until it was full. after that the cuvette was pressed using a hydraulic bench press. inserted the cuvette into boiling water for 5 minutes. The cuvette was opened and the wax fluid was taken outside and the mold was cleaned until there was no more wax leftover.

Put Acrylic resin powder and liquid with 3: 1 ratio into porcelain pot and stirred for 3 minutes until it changed into dough phase. Mold surfaces smeared with could mold seal filled with acrylic resin dough, then it was coated with cellophane plastic. The cuvette was closed and pressed using hydraulic bench press, after that the cuvette was opened and the acrylic excess was removed by using wax knife. The cuvette was closed again, the all sides were pressed together. The cuvette containing heat cured acrylic resin was put into a

pot containing 15 liters of water at 70 degrees Celsius for 20 minutes, and boiling water at 100 degrees celcius for 20 minutes. The fire was turned off and till the cuvette reached room temperature. The acrylic sample was taken and trimmed using bur stones, and then it was smoothed with sandpaper number 320, 500, 800, 1000, and 1500. the sample was polished by using pumice, finally, it was washed with water and incubated with saline solution at 37 degrees Celsius for 24 hours to remove remaining monomers.

The extraction of bay leaves was made by maceration technique, ie: 1.5 kg of bay leaves cleaned and dried in a drying cabinet of 45 degrees Celsius for 48 hours, then dried bay leaves was processed into powder with pollinator and then filtered with a 1mm diameter filter. The bay leaves powder was then mixed with methanol, stirred for 30 minutes, let it for 24 hours, after that it was filtered 3 times. The process would produce filtrate and pulp. Filtrate was evaporated with vacuum rotary evaporator water bath heater of 70 degrees Celsius. Evaporation would produce a thickened extract. It then was put into a porcelain and heated with water bath of 70 degrees Celsius while continuously stirred to get extract of bay leave. Bay leaf extract was diluted with aquadest until it reached 25% concentration which wass used to immerse denture bases of heat cured type acrylic resin.

Denture bases immersion was divided into 6 groups. The first and second groups were immersed into bay leave extracts of 5.5% for 5 days and 15 days. The third and fourth groups were immersed into chlorhexidine gluconate 0.2% for 5 days and 15 days. The fifth and sixth groups were immersed into the aquadest for 5 days and 15 days. The immersion solution was changed daily (24 hours) and the substitution of the artificial denture bases immersion solution was washed with aquadest.

The measurement of surface roughness of acrylic resin bases was done by using surface roughness tester. The tip of the stylus was cleaned by using a cotton swab moistened with alcohol. Before measurement, the sample was placed on the table parallel to the end of the stylus and the tip of the stylus was run. Surface roughness measurements were performed 3 times of the sample surface in a vertical, horizontal and diagonal manner. The results were recorded and averaged with units of **um.**

The data obtained were tested for normality with Saphiro-Wilk test (p> 0,05), then tested homogenity of variance Levene's test and analyzed by using One-Way Anova parametric test. After Anova One-Way analysis, continued with Bonferroni post-hoc.

RESEARCH RESULT

The result of the average calculation of roughness value of heat cured acrylic resin and standard deviation can be seen in table 1.

Tabel 1.the average inhibit zone and deviation standard of antifungal effectivity of garlic extracts with concentration of 2,5%, 5%, 10%, 12,5% and 15% compared with alkaline peroxide towards the grotg of *Candida albicans* at *acrylic heat cured.*

group	repetition (n)	average (mm) ± SD
EBP 2,5%	5	11,296 ± 0,0384
EBP 5%	5	13,256 ± 0,0120
EBP 10%	5	16,308 ± 0,0254
EBP 12,5%	5	18,258 ± 0,0239
EBP 10%	5	19,454 ± 0,0223
Alkalin Peroksida	5	15,330 ± 0,0239

Keterangan :
EBP: garlic extracts
AP: Alkaline Peroxide\

According to table 1, the measurement results showed the acrylic resin roughness value by immersion of 25% 5 days (0.45 + 0.019um) bay leaves extract had a lower surface acrylic resin-surface roughness value than 0.2% 5 days chlorhexidine gluconate solution (0.53 + 0,024 **µm**), a 5%-45% moistened bay leaves extract of 0.45 ± 0.019) causes higher surface roughness of acrylic resin than 5-day aquadest as control (0.37 ± 0.015um) and 0.2% gluconate chlorhexidine solution 5 days (0.53 + 0.024um) causes a greater surface roughness acrylic resin value compared with aquadest 5 days as control (0.037 + 0.015**um**).

The average acrylic resin roughness value after immersion with bay leaves 25% 15 days of (0.55 + 0.012) had lower surface roughness value of acrylic resin compared with 0.2% chlorhexidine gluconate solution 15 days (0, 64 + 0.012). 25%

bay leaves extract 15 days (0,55 + 0,012) caused higher surface roughness of acrylic resin than 15 days aquadest as control (0.43 + 0.013) while 0.2% chlorhexidine gluconate solution 15 days (0.64 +0.012) had larger surface acrylic resin roughness value than the 15 days aquadest as a control (0.43 + 0.013). Analysis continued by looking at differences among treatment groups. Based on Bonferroni Post Hoc test, the results can bee seen in table 2 below.

Tabel 2. Hasil uji Parametrik *One Way ANOVA* efektifitas antijamur ekstrak bawang putih dibandingkan alkalin peroksida terhadap pertumbuhan *Candida albicans* pada *heat cured* akrilik.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	232,480	5	46,496	309,276	,000
Within Groups	3,608	24	,150		
Total	236,088	29			

Tabel 2 menunjukkan hasil uji *One Way ANOVA* diperoleh nilai p=0,000 (p<0,05) bahwa terdapat perbedaan bermakna dari aktivitas perlakuan ekstrak bawang putih dan alkalin peroksida terhadap pertumbuhan jamur *Candida albicans,* kemudian data dilanjutkan dengan *Post Hoc Bonferroni* untuk mengetahui perlakuan yang mempunyai perbedaan efektivitas dengan tingkat kepercayaan 95%. Ekstrak bawang putih 2,5%, 5%, 10%, 12,5% dan 15% memiliki efektivitas antijamur terhadap *Candida albicans*. Hasil uji *Post Hoc Bonferroni* dapat dilihat pada Tabel 3.

Tabel 3. *Post Hoc Bonferroni test antifungal* effectifity of garlic extracts with concentration of 2,5%, 5%, 10%, 12,5% dan 15% compared with alkaline peroxide towards the growth of *Candida albicans* at *acrylic heat cured.*

concentration		p
EBP 2,5%	EBP 5%	0,000*
	EBP 10%	0,000*
	EBP 12,5%	0,000*
	EBP 15%	0,000*
	Alkalin Peroksida	0,000*
EBP 5%	EBP 10%	0,000*
	EBP 12,5%	0,000*
	EBP 15%	0,000*
	Alkalin Peroksida	0,000*
EBP 10%	EBP 12,5%	0,001*
	EBP 15%	0,000*
	Alkalin Peroksida	0,008*
EBP 12,5	EBP 15%	0,000*
	Alkalin Peroksida	0.000*
EBP 15 %	Alkalin Peroksida	0,000*

Keterangan :
EBP: garlic extracts
AP: Alkaline Peroxide

Table 3 show the result of *Post Hoc Bonferroni* test that there is a significant difference of all treatment groups of garlic extracts with concentration of 2,5%, 5%, 10%, 12,5%, 15% and alkaline peroxide with value of p<0,05. Comparison result of inhibit zone between alkaline peroxide and garlic exstracts with concentration of 10%, 12,5%, 15% showed that garlic extracts is better than alkaline peroxide in inhibiting the growth of *Candida albicans***.**

DISCUSSION

This study used 5 days and 15 days periods of immersion, equivalent to immerse 20 minutes per day for 1 year and 5 years. Dentures generally show changes and causes discomfort within 1-5 years so it need denture replacement. According to ADA specification (American Dental Association) no. 12, 17 days immersion is saturation time of heat cured type of acrylic resin in absorbing liquid. The results showed that the longer the immersion time, the higher the roughness value on the acrylic resin

The immersion of denture bases of acrylic resin in any solution may affect the value of its roughness. This is due to physical properties of acrylic resin that is capable of absorbing water so that the solvent particles penetrate and affect the chemical chain of the acrylic resin. The longer the immersion period, the more particles of solution penetrate into microporosity space of acrylic resin. Water molecules penetrate the mass of poly

(methyl) methacrylate and occupy a position between the polymer chains so that this polymer chain separates. This separating polymer chains weaken the chemical structures of acrylic resins. The acrylic resin will be decreased because of its nature to be more plastic. Acrylic resin has ability to absorb water slowly over a certain period of time with a mechanism in accordance with the diffusion law.

Water absorption is primarily happened due to the polar nature of the COOH (carbonyl) group on the acrylic resin. Diffusion occurs between macromolecules causing the macromolecules to be separated from each other. This causes decreasing durability and damage of the surface of the acrylic resin bases over time.

Acrylic resin is a polymer having two kinds of chains, namely primary chain and secondary chain. The primary chain is the chain among the atoms in the chain whereas the secondary chain is the chain among the chains. The primary chain of the acrylic resin has a good durablity while the secondary chain has not. This secondary acrylic resin chain causes water molecule penetrate among the polymer chains through a process known as water absorption. The ability of acrylic resin to absorb water has positive and negative effects. The positive effect is the reinforcing compensation that occurs during the manufacture of denture bases. The negative effect is degradation due to hydrolysis of the polymer. Water-borne ions cause the polymer chains to break down quickly. Weak secondary chain is the main factor of the poor chemical resistance of the acrylic resin surface.

The wider the chemical structure open the weaker its durability.. Such as acrylic resin which has rough surface will more easily absorb water so that its power is reduced. This allows chemical particles to penetrate and break down the secondary chains of the material. Another factor affect the roughness of a heat-cured acrylic resin type is its chemical content, the ratio of powders and liquids that are not fully polymerized. Roughness values in excess of 0.2 **µm** would have potential for plaque buildup and microorganisms. Polishing acrylic resin with pumice to get roughness value of 0.2 um is for 2 minutes.

Bay leaves have chemical content of tannins, flavonoids, 0.05% essential oils consisting of eugenol, and citral and phenol content in extracts of bay leaves varies with percentage of 4.40% - *, 56%. Chemical content is an active ingredient that has pharmacological effects. Content of bay leaves influences the roughness of acrylic resin surface ie: phenol and tannin. Phenol are chemical compound capable of penetrating into an acrylic resin and affecting its mechanical strength. Phenol is a carbolic acid found in bay leaves. It can erode the surface of the acrylic resin because the acid is erosive and abrasive.

Phenol penetrate the surface of the acrylic resin. The ester compound exists in the acrylic resin will be hydrolyzed because of the acid of phenol. The hydrolyzed polymer will be degraded and the acrylic resin strength will be decreasing. Comparative study of heat-cured acrylic resin immersion in artificial saliva for pH 5.5 and 6.8 shows that acrylic resin immersed in artificial saliva with higher pH indicates higher roughness.

Chlorhexidine is a solution based on chlorine (Cl2) and alcohol. Chlorhexidine is bacteriostatic and bactericidal but is acidic. The acid contained in chlorhexidine causes the erosion of the acrylic resin surface because the acid is erosive and abrasive. The color changes and roughness on the surface of the acrylic resin due to the cation and anion interaction of chlorine (Cl2) contained in chlorhexidine with acrylic, so that the acrylic dye fades and there is a surface roughness caused by the alcohol content in chlorhexidine.

The cation reacts with a negative charged of acrylic surface, both of which will bind each other. After the cation is absorbed into the acrylic surface wall, it will destroy the integrity of the acrylic resin surface and continuously release negative ions from the surface of the acrylic resin. The integrity of the affected acrylic surface causes destruction of the intracellular components of the acrylic resin. Such damage leads to the inclusion of anion and alcohol components thus continuously destroying acrylic intracellular components. In conclusion, the effect of 25% bay leaves, 0.2% gluconate chlorhexidine and aquades as denture cleansers for 5 days and 15 days may affect the roughness value of the heat cured acrylic resin surface. immersing effect of 25% bay leaf extract was lower than 0.2% chlorhexidine gluconate to the roughness value of heat cured acrylic resin surface .

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