

# THE EFFECT OF IMMERSING BAY LEAF 25% ON THE MEAN SURFACE ROUGHNESS ACRYLIC RESIN TYPE HEAT

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Denture is a kind of prosthesis which replaces some or all of the original missing tooth and surrounding tissue. Denture basess is a part of denture that rests on the support tissue and where the denture is attached. One of denture basess material used is heat cured acrylic. Heat cured acrylic (Polymethyl methacrylate) has been introduced since 1937. Acrylic resins have non-toxic, non-irritating, insoluble in oral fluid, good esthetics, and easily manipulated.

Heat cured acrylic bases surface of denture is the ideal place for denture plaque formation. Non-cleansed teeth can increase the accumulation of plaque by microorganisms. One of the microorganisms found in denture plaque is the fungus *Candida albicans*. *Candida albicans* is a normal microflora found about 30-50% in oral

cavity and found more in denture users ranging from 60% to 100%. *Candida albicans* penetrate acrylic heat cured, grow and expand on denture surfaces and infect soft tissue, continuous or uninterrupted use for years may cause denture stomatitis. Denture stomatitis is an inflammatory reaction in the soft tissues supporting denture. Denture stomatitis in denture users amounts to 68 respondents (83.95%).

Denture cleansing can be done mechanically, chemically, and combinations of both. Mechanical cleaning is done by using toothbrush and ultrasonic while chemical cleaning is done by immersing denture in disinfectant solution, one of the disinfectant solution is alkaline peroxide in effervescent tablet form used for 5 minutes. Alkaline peroxide has an antifungal effect on *Candida albicans*. Alkaline peroxide removes

stains by releasing oxygen but it may cause roughness and discoloration of denture bases.

The use of denture cleansing materials made of chemicals is relatively expensive, so we need an alternative of cheaper materials. Garlic (*Allium sativum* Linn) is one of the alternative materials which can be used for denture cleanser, it is known as anti-Candida. Garlic contains allicin, saponins, flavonoids, essential oils, and tannins that affect membrane permeability and disrupt the synthesis of fungal nucleic acids. Garlic extracts of 2.5%, 5%, 10% concentrations are able to inhibit the growth of *Candida albicans*. The minimum inhibitory level of garlic extract on *Candida albicans* growth was 2.5% concentration. Based on the facts, this research aimed at analyzing the effectiveness of inhibitory power of garlic extract (*Allium sativum* Linn) with concentrations of 2.5%, 5%, 10%, 12.5% and 15% compared with alkaline peroxide to the growth of *Candida albicans* on the surface of heat-cured acrylic.

## MATERIALS AND METHODS

This research used pure experimental laboratory method (true experiment) with post-test only with control group design and randomized design using 7 treatment groups. Each treatment group was given garlic extract 2.5%, 5%, 10%, 12.5%, 15%, alkaline peroxide as positive control, and aquadest as negative control. The number of repetitions for each treatment was 5 times based on the Federer formula. This research was conducted in June-November 2016 in Skills Room of Laboratory of Dentistry Faculty and Microbiology Laboratory of Medical Faculty of Universitas Lambung Mangkurat.

The materials used in this study were heat-cured acrylic, white cast, blue cast, red wax, aquadest, saline solution, garlic extract of 2.5%, 5%, 10%, 12.5%, 15% Alkaline peroxide, Sabouraud Dextrose Agar (SDA), *Candida albicans* suspension, Brain Heart infusion (BHI) media, sterile blank disc paper, McFarland solution sequence and Could Mold Seal (CMS). The tools used were Bowl, Spatula, Tweezers, knives, cuvettes, test tubes, measuring cylinders, vibrators, Hydraulic Bench Press, stoves, pans, autoclaves, incubators, petri dishes, bunsen, ose, cotton sterile, calliper, Erlenmeyer, aluminum foil, sandpaper number 600 and 1200, bur stone, and straight handpiece.

The research procedure began with the manufacture of 7 pieces of heat-cured acrylic plate

made of 10mm x 10mm x 2mm size by using red wax. The cast mixture was made of 75 ml: 250 gram water and cast ratio, stirred in a rubber bowl with a spatula for 60 seconds and put into a prepared lower cuvette then be vibrated. Red wax laid on the dough and let it for 15 minutes. The casts surface in the lower cuvette was smeared with vaseline and the upper cuvette was fitted, which was then given cast dough (done while in vibration). When the cast was being hardened, cuvette was opened and mold was taken out or wax was poured with hot water until it was clean. When it became clean, then we got mold space from red wax mold. The acrylic heat-cured plate material was stirred in a mixing jar with a powder and fluid ratio of 6gram: 3ml at room temperature (28°C). The resin dough reached a certain turbidity level according to McFarland's standard 0.5 (108Cfu / ml).

Here are steps in making garlic extract; first, fresh 100gr garlic is peeled, wash thoroughly and blend with 50ml aquadest, mix it homogeneously. After garlic juice is mixed well, then it is centrifuged at 3000 rpm for 30 minutes and filtered using Whatmann no. 1, so that garlic extract is obtained. The extract of garlic yields 40.7% concentration. Then dilute the extract solution according to the concentration that will be used i.e.: 2.5%, 5%, 10%, 12.5%, and 15% by measuring the volume, then dilute with aquadest.

*Candida albicans* isolates were obtained by breeding process at the Microbiology Laboratory of the Faculty of Medicine, Universitas Lambung Mangkurat Banjarmasin. *Candida albicans* isolates cultured were taken with sterile ose, dissolved into 10ml liquid BHI medium, being incubated for 8 hours at 37°C to obtain *Candida albicans* suspension. The *Candida albicans* suspension was diluted by adding aquadest to achieve a certain turbidity level according to McFarland's standard 0.5 (108cfu / ml).

The study used diffusion method by preparing the acrylic heat-cured plate with 10mm x 10mm x 2mm size as many as seven plates immersed with aquadest for 48 hours to remove the remaining monomer, then it was taken with sterile tweezers, it was immersed in saline solution for about one hour. Acrylic was put outside from saline solution, immersed it in the reaction tube containing 10 ml of *Candida albicans* suspension. BHI medium adjusted to McFarland standard 0.5 incubated for 24 h at 37 ° C. Seven acrylic plates were divided into seven groups and immersed for 5 minutes in

seven groups of garlic extract treatment with concentrations of 2.5%, 5%, 10%, 12.5%, and 15%, alkaline peroxide and aquadest. The acrylic heat cured plate was taken outside each treatment group and rinsed with saline. The acrylic plate was inserted into a reaction tube containing BHI and then vibrated for 30 seconds so that *Candida albicans* fungus attached to the acrylic heat cure plate and incubated for 8 hours. *Candida albicans* isolate was wiped to each SDA media in petri dish.

Paper disk immersed into seven groups of garlic extract treatment 2.5%, 5%, 10%, 12.5%, 15%, alkaline peroxide and aquadest for 3 hours, then inserted the paper disk in each media SDA that had been wiped with isolated *Candida Albicans* and incubated for 24 hours. The inhibit zone formed at all treatments was measured using a calypher in millimeter units.

### RESEARCH RESULT

The results of the study "The Effect of antifungal of garlic extract compared with alkaline peroxide on the growth of *Candida albicans* on the surface of heat cured acrylic". The treatments which had been tested were garlic extract with concentration of 2.5%, 5%, 10%, 12.5% and 15% and alkaline peroxide. Based on the measurement of the inhibitory zone of each treatment on the growth of *Candida albicans* at medium of test, we obtained the average and deviation standard which can be seen in Table 1.

Table 1. the average of inhibitory zones and deviation standard of the effectiveness of antifungal from garlic extracts concentrations of 2.5%, 5%, 10%, 12.5% and 15% compared with alkaline peroxide on the growth of *Candida albicans* on the surface of heat cured acrylic.

Kelompok	Mean ± SD (µm)
Klorheksidin 5 hari	0,53 ± 0,024
Klorheksidin 15 hari	0,64 ± 0,012
Ekstrak daun salam 5 hari	0,45 ± 0,019
Ekstrak daun salam 15 hari	0,55 ± 0,012
Akuades 5 hari	0,37 ± 0,015
Akuades 15 hari	0,43 ± 0,013

Table 1 shows the effect of garlic extract treatment with concentrations of 2.5%, 5%, 10%, 12.5% and 15% respectively resulting the average inhibit zone of 11.29mm, 13.25mm, 16.30mm, 18.25mm, and 19.46mm. The alkaline peroxide

treatment effect yielded the average inhibit zone of 15.33 mm. Garlic extract at concentrations of 10%, 12.5%, 15% had a higher inhibitory zone than alkaline peroxide in inhibiting the growth of *Candida albicans*.

All data were tested for normality of Saphiro-Wilk and it was found that all data were normally distributed  $p > 0,05$ , then the homogeneity of data was tested using Levene's test. It was found that all data was homogeneously distributed. One way ANOVA test was conducted to find out whether there were significant differences between treatments tested with 95% confidence level. Data One Way ANOVA test results can be seen in Table 2.

Table 2. One Way ANOVA test results of antifungal effectiveness of garlic extract compared with alkaline peroxide to the growth of *Candida albicans* on heat cured acrylic.

Kosentrasi		p
Ekstrak daun salam 25% 5 hari	Klorheksidin gluconat 0.2% 5 hari	0.000*
	Akuades 5 hari	0.000*
Ekstrak daun salam 25% 15 hari	Klorheksidin gluconat 0.2% 15 hari	0.000*
	Akudes 15 hari	0.000*
Klorheksidin gluconat 0.2% 5 hari	Akuades 5 hari	0.000*
Klorheksidin gluconat 0.2% 15 hari	Akuades 15 hari	0.000*

Table 2 shows One Way ANOVA test results  $p = 0,000$  ( $p < 0,05$ ) in which there was a significant difference activity of extract treatment of garlic compared with alkaline peroxide to the growth of *Candida albicans* fungus, then data was continued with Post Hoc Bonferroni to find out the difference of treatment in effectiveness with 95% confidence level. Garlic extracts 2.5%, 5%, 10%, 12.5% and 15% had antifungal effectiveness against *Candida albicans*.

### DISCUSSION

Garlic extract of 2.5%, 5%, 10%, 12.5%, and 15% have antifungal effectiveness on *Candida*

albicans growth. Garlic consist of 33 sulfur compounds, 17 amino acids and minerals. The contents of antifungal of garlic are allicin, saponin, flavonoids, essential oils and tanin. This study uses aquadest to make the extract of garlic because it can bind the active compound in garlic extract. Garlic extract is better in binding active substances of garlic compared with the use of botanicals or extracts using cloroform solvent, acetone and ethanol. Compounds that can be binded by aquadest are anthocyanins, starches, saponins, tannins, terpenoids, polypeptides and lectins. The use of aquadest in extracting process of garlic ties up the compound of Allicin, tannin and saponins.

Alliin in a fresh garlic (diallylthiosulfinate or diallyl disulfide) is one of the most active compounds biologically. Alliin in garlic have antimicrobial effects that can fight viruses, bacteria, fungi and parasit. Alliin shows antimicrobial activity by inhibiting the synthesis of RNA, synthesis of DNA and protein synthesis from microorganisms.

The sulfur compounds which are most numerous in garlic is alliin (S-allylcysteine sulfoxide) which are respectively at 10 and 30 mg / g in fresh and dry garlic. Alliin is found through enzymatic reaction after cutting or crushing the garlic. Cutting or crushing garlic activates the enzyme alliinase which changes alliin into allicin. alliin is responsible for the aroma and flavor of garlic, sulfur-containing amino acid and is used as a precursor all<sup>5</sup>n. Precursor alliin is a non-proteinogenic amino acid alliin (S -allyl-L-cysteine sulfoxide) . Alliin and sulfoxide S-alkyl-L-cysteine which is hydrolyzed by the enzyme alliinase. Garlic which is dissolved with water decomposes into diallylsulfide, diallyl disulfide and diallyltrisulfide which later merged into the organic polysulfide.

Other compounds contained in garlic are tannins and saponins. Tanin is a complex binding compound in the form of polyphenols capable of reacting with cell walls and inhibiting chitin cells of Candida albicans, and tanins can also activate microbial cell attachment that exists in cell wall polypeptides. Tannin is a phenol compound. Saponins have antifungal effect on Candida albicans by destroying the cell membrane of the fungus. Saponins as surfactants has the ability to crush the fatty layers of cell membranes which causes impaired cell membrane permeability and disturb the diffusion process of substances required by fungi. Saponins and tannins found in garlic interact with the cell surface of the fungus through

hydrogen chains at certain concentrations and temperatures and cause hydrogen chains to be weak.

There is an increasing garlic extract concentrations of 2.5%, 5%, 10%, 12.5%, 15%. The higher concentration of garlic extract the stronger antifungal effect and also the larger the inhibitory zone diameter. The lower concentration of garlic extract, the weaker antifungal effect and the smaller the inhibitory zone diameter, as seen in the difference inhibitory zone diameter.

The compounds contained in the alkaline peroxide is useful to destroy and oxidize the cell wall of microorganisms. This reaction causes the dissolved tablet put into water decompose sodium perborate and forms alkaline peroxide, this compound releases oxygen in which called the process of mechanical and chemical cleaning of denture bases. The ability of oxidation and decomposition of peroxide as the result of effervescent reaction produces oxygen. Sodium carbonate works through the process of releasing oxygen bubbles. The oxidation of H<sub>2</sub>O<sub>2</sub> releases oxygen, which provides a mechanical cleansing effect from releasing the oxygen bubbles. Oxygen bubbles can effectively remove organic deposits and kill microorganisms. Alkaline peroxide is unable to remove biofilm completely whereas biofilm is the place of colonization and infection of Candida albicans.

The conclusion of this research is there are different inhibitory effects between garlic extract concentration of 2.5%, 5%, 10%, 12.5%, 15%, and alkaline peroxide on the growth of Candida albicans on heat cured acrylic. Garlic extract concentrations of 10%, 12.5%, and 15% have greater inhibitory effect than alkaline peroxide on Candida albicans growth. It needs further research on the side effects of garlic extract on color change, roughness, and impact strength on acrylic heat cured and how to improve the stability of garlic extract to last longer.

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