Antifungal Activity of Freshly Squeezed Garlic as Denture Cleanser on *Candida Albicans* Growth

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Abstract

The objective of this study was to investigate the growth of *Candida albicans* on acrylic resin plate after soaking in freshly squeezed garlic. Samples were immersed in sterile saliva for 1 hour at room temperature for the formation of pellicle. They were taken and rinsed with phosphate buffer saline (PBS) solution, contaminated with *Candida albicans* by insertion into a tube containing *Candida albicans* suspension, incubated for 24 hours at 37^0 C, removed and rinsed with PBS solution, soaked in freshly squeezed garlic in test tubes for 3 minutes, containing 10 ml of freshly squeezed garlic of 20%, 30 %, 40 % concentration each and sterile distilled water. Samples were taken and rinsed with PBS, inserted into Sabouraud's broth, vibrated with vortex for 30 seconds to release *Candida albicans* attached to them. Suspension from Sabouraud's broth was taken, dripped on Sabouraud's dextrose agar, spreading, incubated for 48 hours, at 37^0C. *Candida albicans* colonies were calculated. Data were analysed using Anova and followed by LSD test.

The Results showed that there were significant difference among the groups ($p < 0.05$). In conclusion, the higher concentration of freshly squeezed garlic the lower the number of *Candida albicans* colonies.

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Introduction

Heat-cured acrylic resins are still the main choice for denture base materials because they are not toxic, easy to manipulate, not irritating, have good aesthetics, color can be made similar to gingiva and stable, easy to repair, small dimensional changes and more affordable price.[1] The teeth and denture in the mouth will always be in contact with the saliva, so the denture is immediately coated by the saliva pellicles.

This layer of pellicle is the adhesive medium of microorganism, similar to the enamel and mucosa of the mouth.[2] Acrylic resin as an artificial denture base is a place that can lead to accumulation of stains, and plaque that will adversely affect the soft tissue health of the oral cavity. The accumulation of food scraps and plaque will cause *Candida albicans* colonies to increase causing increased product of endotoxin of *Candida albicans* and will penetrate into mucous membrane and cause inflammation called denture stomatitis. The patient who smokes will have the potential to increase C albicans virulence and increase the biofilm formation by Candida albicans.[3] Still, elderly patients frequently suffer from poor oral hygiene, which is a major aetiological factor in the pathogenesis of denture stomatitis. In addition to that, elderly patients with reduced general condition have a higher risk of developing denture stomatitis.[4]

Denture stomatitis caused by *Candida albicans* infection can be prevented by keeping oral hygiene, maintaining and cleaning the denture and removing it at night. Some ways are used to maintain denture hygiene, those were mechanically and chemically. Mechanical cleaning is done by using a toothbrush, while chemical cleaning is done by immersion of denture in an artificial tooth cleaning solution for

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15-30 minutes depending on the material used.[5] Chemically denture cleanser greatly helps aged denture users who are mostly elderly with all the limitations to be less careful in cleaning the denture. Currently, many types of artificial tooth cleaning solutions are on the market with a relatively expensive price. One alternative that people can use a cleaning solution that is affordable is to use garlic as a denture cleanser. Garlic contains sulfur such as allicin (diallyl-dithiosulfinate) which has antifungal activity especially against *Candida albicans.*[6]

Based on the above description it is necessary to investigate the antifungal activity of freshly squeezed garlic as a denture cleanser on the growth of *Candida albicans*.

The aim of this study is to investigate the growth of *Candida albicans* on acrylic resin after soaking in freshly squeezed garlic at 20%, 30% and 40% concentration.

Materials and Methods

Twenty eight specimens (10 x 10 x 1 mm) of *heat-cured* acrylic resin material (QC-20, *cross linked*, de Trey, England) were fabricated according to manufacturer's recommendations.

All the wax patterns were invested with a dental stone in metallic flasks. After the setting of stone, the flask helves were separated, the wax was removed, and the stone mold was cleansed. The resin was manipulated packed and pressed into the mold according to the manufacturer's instructions. The heat polymerization method was in water at 100 °C for 20 min. All flasks were allowed to cool to room temperature before opening. After polymerization of the resin, the specimens were removed from the molds and immersed in distilled water at 37 ± 1 °C for 48 hours for residual monomer elimination. The excess resin was trimmed with a tungsten steel bur (Maxicut, Malleifer SA, Switzerland) using a handpiece at low speed.

Freshly squeezed Garlic (Allium sativum Linn) preparation : 25 grams of garlic to get freshly squeezed garlic as much as 2 ml with 100% concentration. The garlic bulbs were washed with sterile distilled water. Garlic press sterilized with 70% alcohol. Garlic bulbs are inserted in garlic press until we obtained crushed and smooth garlic then wrapped with sterile gauze and squeezed. The freshly squeezed Garlic obtained has 100% concentration. To get 20% concentration, freshly squeezed garlic as much as 2 ml diluted with 8 ml sterile distilled water, it will get 20% freshly squeezed garlic as much as 10 ml. To get 30% concentration, 3 ml freshly squeezed garlic diluted with 7 ml of sterile distilled water, similarly to get the concentration of 40%, as much as 4 ml freshly squeezed garlic diluted with sterile distilled as much as 6 ml.

The study consisted of 1 group soaked in sterile distilled water as a control (A), 3 groups of treatment that were the group soaked in freshly squeezed garlic at 20% (B), 30% (C), 40% (D) concentration., each group consisted of 7 samples. Samples of acrylic plate were sterilized in 121^0 C autoclave for 15 minutes.[7]

The samples were immersed in sterile saliva for 1 hour at room temperature for the formation of the pellicle. Sterile saliva is collected from one person. Saliva collected was centrifuged at 1000 rpm for 20 minutes at 4^0C. Samples were taken and rinsed with phosphate buffer saline (PBS) solution twice. Samples were contaminated with *Candida albicans* by insertion into a tube containing *Candida albicans* suspension and incubated for 24 hours at 37^0 C.

The samples were removed and rinsed with PBS solution twice and soaked in freshly squeezed garlic in test tubes for 3 minutes, each test tube containing 10 ml of freshly squeezed garlic of 20%, 30 %, 40 % concentration, and sterile distilled water (control group).

Samples were taken and rinsed with PBS twice, inserted into 10 ml Sabouraud's broth, vibrated with vortex for 30 seconds to release *Candida albicans* attached to the samples. Ten millilitre Suspension of *Candida albicans* from Sabouraud's broth was taken 0.1 ml using tuberculin syringe, dripped on Sabouraud's dextrose agar, spreading, incubated for 48 hours, at 37^0C, then *Candida albicans* colony were calculated (CFU / ml).[8]

Statistical analysis. The data obtained from this research expressed as mean ± standard deviations (SD). One-way analysis of variance (ANOVA) followed by LSD test was applied to assess the statistical significance of the differences between the study group at $p<0.05$.

Results

Data were presented in Table 1. Based on the results of observation and calculation on *Candida albicans* colonies on control group that were immersed in sterile distilled water and 3 groups of treatments that were immersed in freshly squeezed garlic at 20%, 30%, and 40% concentration.

The results of *One way Anova* test showed that there were significant differences in the number of Candida albicans colonies between the groups (p = 0.000). Thus, statistical analysis was continued with LSD test. The number of Candida albicans colonies in the group A, B, C and D were presented in Figure 1.

Group	N	Mean	SD
A(Control)	7	256.5714[a]	10.95228
B (20% concentration)	7	162.1429[b]	21.80542
C (30% concentration)	7	125.0000[c]	8.71780
D (40% concentration)	7	87.8571[d]	3.71612

Table 1. Mean and SD of *Candida albicans* colonies number in control and treatment group. [a,b,c,d] Different superscript letter show a significant difference ($p<0.05$)

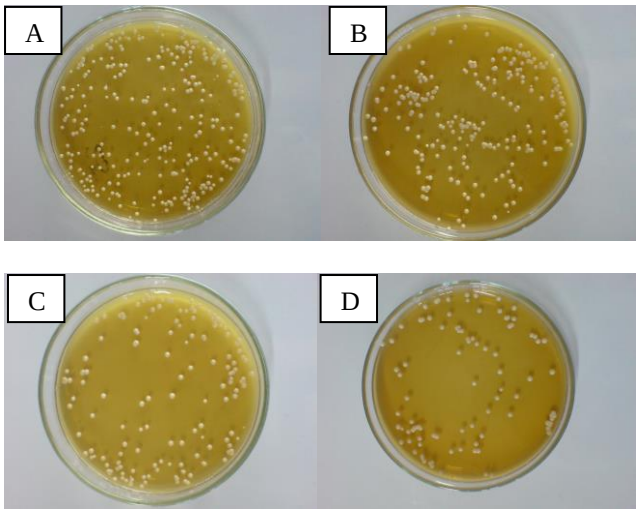

Figure 1. Petri dish photos of antifungal activity assay of freshly squeezed garlic with different concentrations (%) against *C. albicans* (A) Control, (B) 20 %, (C) 30 % and (D) 40 %.

Discussion

One of the removable denture components is an acrylic denture base. This acrylic denture base is in direct contact with the oral mucosa. Continuous denture usage can cause the mucosa beneath the acrylic denture base to close for long periods of time, thus blocking the mucosal clearance. Continuous mucosal closure is a predisposing factor in the onset of chronic athropic candidiasis. Proliferation of fungi can cause bad breath, acrylic resin color change and staining, calculus formation and chronic atrophic Candidiasis known as denture stomatitis.[9] *Candida albicans* can colonize and cause infection because it has the ability to stick to the surface of the epithelium, similar to the fitting surface of the denture and as a source of infection.[10]

Unfinished denture surfaces (fitting surface) can facilitate plaque attachment and is a good place to settle germs. Oral inflammation often found under maxilla dentures.[11] Earlier researchers reported that biofilm denture serves as a source for opportunistic microorganisms that can cause local infection, especially *Candida*-related denture stomatitis.[12] Denture stomatitis is an infectious *Candida* infection in the mouth most commonly found in the denture user's palate.[13]

The alliin (S-alilcisteína S-oxide) is the main organsulfur ingredients identified in intact garlic bulbs, as they are cut or grinded, the alliin is changed to allicin by alliinase.[14] Allicin is the main component of freshly squeezed garlic, being attributed to it the most of its biological activities, such as bactericidal, antifungal and antiviral actions.[15,16] Garlic has been shown to inhibit growth of fungal diseases, as equally as the drug ketoconazole, when tested on the fungi *Malassezia furfur*, *Candida albicans*, *Aspergillus, Cryptococcus* and other *Candida* species.[17]

The results of this study showed a significant difference in the amount of *Candida albicans* colonies on acrylic resins that have been soaked in freshly squeezed garlic concentration of 20%, 30%, and 40% with the control group. This study shows that 20% concentration of freshly squeezed garlic has been able to inhibit the growth of *Candida albicans*. The average value of *Candida albicans* colonies on the base of denture acrylic soaked in garlic concentration 20% was 162.1, 30% was 125.0, 40% was 87.8. This study illustrates that the higher

concentration of freshly squeezed garlic the lower the number of *Candida albicans* colonies. The higher concentration of freshly squeezed garlic, the more content of Allicin so that the effectiveness to inhibit *Candida albicans* growth is higher. This is due to the content of allicin (diallyl thiosulfinate) in the freshly squeezed garlic that serves as the main antifungal, among the various organosulfur compounds that are formed.[18] Allicin is the single and most dominant antifungal component of the main and bioactive compounds present in freshly squeezed made garlic.[16] Allicin has fungistatic and fungicidal effect and destroys the physical structure of fungal cell wall.[15] Allicin may inhibit the proliferation of fungal cells or kill the cells directly depending on the dose,[19] according to this study which proves that the higher the concentration the lower the growth of *Candida albicans*. In addition to inhibiting the proliferation of fungal cells, allicin also inhibits the production of cell wall degrading enzymes *Candida albicans*, consequently the colonization of *Candida albicans* decreased significantly.[20] Most fungi use various cell wall degrading enzymes for penetration into host cell compartments.[21] and if this enzyme is controlled or disturbed, then the ability to penetrate into the host body is disrupted and causes colonization inside the host to be disrupted.

The organosulfur compounds such as diallyl disulfide (DADS), diallyl trisulfide (DATS), Ajoenes, may contribute to biological activity as a garlic derived compound,[22] but during the decomposition of allicin into other compounds (DADS, DATS, Ajoenes), the anti-bacterial properties decreased.[19] Therefore the antifungal potential of freshly made garlic and directly used as an anti-fungus, used in this study can be attributed primarily to the contribution of allicin content.

Conclusions

The conclusion of this study was the immersion of heat cured acrylic plate in the freshly squeezed garlic at concentration of 20% decreased the number of *Candida albicans* colonies and the higher the concentration of the freshly squeezed garlic, the lower the number of colonies *Candida albicans.*

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