# Denture adhesive antifungal potency towards the growth of *Candida albicans*

**Siti Utami Dewi*), Taufik Sumarsongko*, Warta Dewi****

***Department of Prosthodontics Faculty of Dentistry Universitas Padjadjaran**
****Department of Oral Biology Faculty of Dentistry Universitas Padjadjaran**

**ABSTRACT**

Denture adhesive is a device that applied to the base of a denture before the denture is inserted to the mouth. The device used to improve denture retention and stabilization. It was thought that added antifungal agent to denture adhesive might be an effective way to inhibit the growth of *Candida albicans* and prevent denture stomatitis.The study was performed as an experimental laboratory study by observed antifungal test of denture adhesive against *Candida albicans* growth using Kirby Bauer diffusion agar method with 5 samples and 3 time repetitions. The conclusion of this study was that the antifungal agent in denture adhesive containing poly (methylvinylether/maleic acid), sodium-calcium mixed partial salt and prophyl hydroxybenzoate and denture adhesive containing sodium carboxymethylcellulose couldn't inhibit the growth of *Candida albicans*.

**Key words:** Denture adhesive, *Candida albicans*

***ABSTRAK***

Denture adhesive *merupakan bahan yang digunakan pada bagian anatomis gigi tiruan sebelum gigi tiruan digunakan didalam mulut pemakai gigi tiruan dengan tujuan untuk meningkatkan retensi dan stabilitas gigi tiruan. Penambahan zat yang memiliki daya antijamur pada* denture adhesive *dianggap sebagai cara efektif untuk menghambat pertumbuhan Candida albican sehingga dapat mencegah terjadinya* denture stomatitis. *Penelitian ini dilaksanakan secara eksperimen laboratoris dengan menguji daya antijamur* denture adhesive *terhadap pertumbuhan Candida albicans menggunakan metoda difusi agar Kirby Bauer dengan sampel sebanyak 5 orang dan pengulangan sebanyak 3 kali. Berdasarkan hasil penelitian disimpulkan bahwa tidak terdapat daya antijamur pada* denture adhesive, *baik yang mengandung poly (methylvinylether/asam maleic)* sodium-calcium mixed partial salt *dan prophyl hydroxybenzoate serta* denture adhesive *yang mengandung sodium carboxymethylcellulose terhadap pertumbuhan Candida albicans.*

***Kata kunci:*** Denture adhesive, *Candida albicans*

**)Correspondence author: Siti Utami Dewi, Department of Prosthodontic Faculty of Dentistry Universitas Padjadjaran Jl. Sekeloa Selatan No. 1 Bandung, West Java-Indonesia, Tel./Fax: +6222-2504985/2532805*

signs on gingival. It is because *Gambir* also belongs to polyphenol compound group, as stated by Yang et al. that polyphenol regulates the excretion of TNF-α gen by the activation modulation of nuclear κB factor through its antioxidant nature. TNF-α stimulates the immune cells in the inflammation process. Catechin may act to prevent inflammation via anti TNF-α. Further studies are still needed to see what bacteria can be inhibited by the use of *Gambir* catechin preparation, and the healing process in infected tissues due to the bacteria and an ideal preparation form for irrigation in subgingival area.[15]

## CONCLUSION

Subgingival irrigation of *Gambir* catechin I and II can decrease the number *A. actinomycetemcomitans* of but there was no statistical difference between the two groups. A significant difference is seen between Group I (aquadest) with Group I and Group II. It shows that catechin can be used as an alternative in periodontitis patients' condition.

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## INTRODUCTION

*Candida albicans* is a common oral microorganisms; estimated to present in 40% of the normal population and are found in 50% patients at the dental hospital.[1,2] *Candida albicans* releases endotoxins that damage the mucosa of the mouth and causes the occurrence of stomatitis in denture users. Siawomihardjo[3] even mentioned that *Candida albicans* is the most dominant species found on the surface of the denture on the patient with stomatitis due to use of denture.

The main candida infection was superficial, such as acute pseudomembranous infection in the mouth or vagina. Currently candidiasis associated with denture use is called Candida-related denture stomatitis. Since 1936, *Candida albicans* has been found in 75% of patients who use or have used denture denture without signs of inflammation and can be found in normal people.[4]

Denture stomatitis is a pathological condition in the oral cavity, usually painless, characterized by clinical symptoms of redness of the soft tissues in the oral cavity, particularly the part that is covered by the prosthesis. Prevention of *Candida* infection is done by inhibiting the growth of *Candida albicans* on the surface of the denture base, such as by using a denture adhesive.[5]

Denture adhesive is a material attached to the anatomical denture surface and the surface of the denture supporting tissues. Denture adhesive can increase the viscosity of saliva and fill in the gaps that exist between the denture base and support network, which can decrease the risk of slipping.[6] The use of denture adhesive is very important in completing the final try-in, because the use of denture adhesive will improve the accuracy of the denture. Denture adhesive also plays a role in a process of adaptation to new patients who use denture because it provides comfort and prevents irritation of the supporting tissues.[7,8]

The use of denture adhesive may reduce the amount of food trapped under the denture and also inhibits the growth of *Candida albicans*.[8] Attachment of microorganisms that occur in the denture base is strongly influenced by the shape of the surface topography of materials, so that more microorganisms such as *Candida albicans* are attached to the unpolished surface such as the anatomical surface.[3]

A survey conducted by Sadamori et al.[9] found that the numbers of dentists in Indonesia who advise their patients to use denture adhesive are more than the number of dentists advising their patients to use denture adhesive in Japan. Ideal denture adhesive should have the ability of attachment over 12-16 hours.[8] Due to the long use of denture adhesive in the mouth, denture adhesive is concerned to be able to act as a media that affect the microorganisms in the mouth resulting in an imbalance of commensal microorganisms in the oral cavity that can lead to pathological states.[1,10] Moreover, the attachment of microorganisms such as *Candida albicans* occurs rapidly after biofilm formation that occurred within 2 hours after denture contact with saliva.[5]

The addition of an antifungal substance in denture adhesive is considered as an effective way to avoid the occurrence of denture stomatitis. The use of denture adhesive containing antifungal agents may serve as a preliminary treatment to prevent the occurrence of denture stomatitis. In addition, denture adhesive containing an antifungal agent plays role in the treatment of denture stomatitis because it may reduce the irritation of the supporting tissues that lead to denture stomatitis and may also help the recovery process because it is applied to the entire surface of a supporting tissues.[5] Therefore, efforts to improve the function of denture adhesive are made by adding ingredients that have antibacterial or antifungal activity.[11]

Denture adhesive used before the beginning of 1960, were made of the simple sap of plants (karaya gum, tragacanth, xanthan gum, and acacia), nonionic adhesion with slight cohesion to the denture. Denture adhesive made of the sap of the plant were not durable and the use of it was unsatisfactory.[6] Currently, the denture adhesive is made using materials that have a bio-adhesive ability and high cohesion, such as *sodium carboxymethylcellulose* (CMC) that has a strong adhesion and *poly (methylvinylether/maleic acid)* (PVM-MA), which has antibacterial and antifungal activity.[7,12,13]

Microbiological analysis were conducted to prove the antibacterial and antifungal activity of *poly (methylvinylether/asam maleic) sodium-calcium mixed partial salt* and resulted in antibacterial activity towards: *Escherichia coli, Proteus*

*mirabilis, Pseudomonas mallei, Staphylococcus epidermidis, Pseudomonas aeruginosa,* and *Salmonela typhii*. The analysis were also resulted in the antifungal activity towards *Candida albicans* and *Aspergillus niger.*[14]

Denture adhesive ability in inhibiting the growth of microorganisms is also contained in substances such as *sodium borate, methylparaben, and prophylparaben (prophyl hydroxybenzoate*) that serves as a preservative.[6] In general, *prophylparaben* is more effective as antifungal than as antibacterial and it has better activity on gram-positive than gram negative microorganisms. *Prophyl hydroxybenzoate* activity is resulted in a separation of cell membranes and membrane permeability damage. This process results in inhibition of growth of microorganisms.[15]

The research of denture adhesive role on the growth of microorganisms was begun, that denture adhesive did not inhibit the growth of microorganisms. In 1950, Grasso et al.[12] reported that denture adhesive does not support bacterial growth. Nearly 20 years later, Stafford and Russell[1] found that the denture adhesive that they test provides support for the growth of some microorganisms (*Candida albicans, Streptococcus mitis*), and they could not prove whether the denture adhesive had the ability to inhibit growth of oral flora. Research conducted by Makihira et al.[16] towards 6 types of denture adhesive showed inhibition of growth of *Candida albicans* by monitoring pH changes in the culture medium. The result showed that the denture adhesive has antifungal activity at a particular level from low to high.[16]

## METHODS

The research was carried out as laboratory experiments to test the effect of antifungal activity in denture adhesive A that contains *poly (methylvinylether/maleic acid) sodium-calcium* partial mixed salt and *prophyl hydroxybenzoate* (GSK) and denture adhesive B that contains *sodium carboxymethylcellulose* (Fittydent International GMBH) on growth *Candida albicans*. The study was conducted at the Laboratory of Microbiology Faculty of Dentistry, Universitas Padjadjaran Jatinangor in August 2010.

The population in this study was *Candida albicans* in anatomical surface of full dentures, and the sample in this study was isolated *Candida albicans* from full denture smears. Population obtained from smears performed in elderly full denture users in *Panti Sosial Tresna Wredha Budi Pertiwi Bandung* and *Panti Sosial Tresna Wredha Asuhan Bunda Bandung* with the following criteria: female, elderly, has used full denture for at least 6 months; and has adequate denture retention and stabilitation.

The tools used for making the examination of materials prepared in a sterile condition. Examination materials (EM) was obtained by taking swabs from five full denture users who have used the denture for six months or more, and then preparations were made directly with Gram staining and viewed under a microscope. Examination material was then inserted into the water and sterile broth as a medium transport. Examination material was taken to the laboratory for further examination. Examination material of 0.1 ml was grown on Sabouraud agar plates and incubated at room temperature of 37° C for 24 hours in facultative anaerobes condition.[17] Cultures grown on Sabouraud agar showed characteristics; round, convex, white-yellowish color and peculiar odor of yeast, and was defined as a suspect colony of *Candida.*[18]

In the colonies that were suspected as colonies of *Candida* spp, microscopic examination were checked with the smears stained with Gram stain to look for pseudohyphae and budding cells.[19] Gram stain showed the characteristic purple color because they were Gram positive, round, spherical or oval shape, and budding yeast cell or a characteristic of "Germ tube" were seen at some colonies. *Candida albicans* cultures grown on some liquid carbohydrates will yeast glucose and maltose to produce acid and gas; acid from sucrose, and no reaction to lactose. Fermentation of carbohydrates, along with the properties and morphology of the colonies, differentiate *Candida albicans* from other *Candida* species.[17]

Fermentation tests performed on the carbohydrates germination: glucose, maltose, sucrose, and lactose which has been supplemented phenol-red as indicator. The color change of the indicator phenol red-red to yellow indicates the formation of acid in the fermentation reaction. To understand the formation of gas, a Durham tube was used in reverse. Gas that was formed will appear as an empty space in the tube.

For antifungal activity test with diffusion method, *Candida albicans* suspension was prepared according to Mc. Farland turbidity standard of 0.5 and incubated for 2 hour at 37°C in facultative anaerobes; 0.1 ml suspension was grown on Sabouraud agar plates and levelled with a cotton stick. On culture, three pieces of 10 mm diameter hole was made with a perforator. A denture adhesive cream that contains *poly (methylvinylether/maleic acid) sodium-calcium*-containing mixed partial salt and propyl hydroxybenzoate (GSK) was inserted into the first hole about one-third the thickness of the agar. Similarly, denture adhesive cream containing sodium carboxy methyl cellulose B (Fittydent International GMBH) was inserted into the second hole. Each of the denture adhesive which inserted into the hole had previously been diluted with artificial saliva ± 0.1 ml and the third hole filled with artificial saliva as a control. Agar plates were incubated for 24 hours at 37°C. The whole steps were repeated three times. After 24 hours, changes in the surface of the agar plates were observed. If the materials tested had antifungal ability on the fungus test, it would show a clear area around the hole, which means there was no fungus growth.

## RESULTS

According to the research, the growth of *Candida albicans* colonies were not found around the hole filled with both *denture adhesive* containing *poly (methylvinylether/maleic acid) sodium-calcium mixed partial salt* and *prophyl hydroxybenzoate* (denture adhesive A) and denture adhesive containing sodium *carboxymethylcellulose* (denture adhesive B) (Fig. 1).

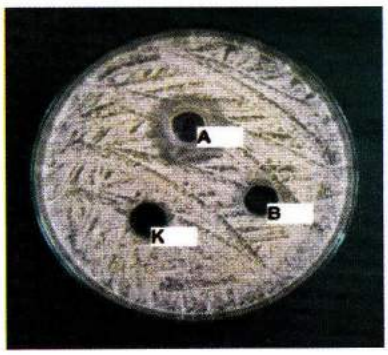


**Figure 1. Antifungal test result with diffusion agar method. Note: A=Denture adhesive A, B=Denture adhesive B, K = Control.**

## DISCUSSION

Trauma caused by the use of denture followed by increasing the number of *Candida albicans* in the oral cavity was believed to be a major factor in denture stomatis.[4] *Candida albicans* releases endotoxins that damage the mucosa of the mouth and causes the occurrence of stomatitis in denture users.[3]

*Candida albicans* will produce proteinase that degrade salivary proteins, including secretory IgA, lactoferrin, mucin and keratin which may deliver a cytotoxic effect towards host. *Candida albicans* also produce phospholipase contained in the hyphae that play a role in the invasion of *Candida albicans* to the host resulting in infection. Therefore, the study was conducted to test the antifungal activity against *Candida albicans* in denture adhesive using Kirby Bauer agar diffusion method by measuring the transparent area around the holes that contained test materials. Area shows antifungal ability possessed by the test materials in the form of a transparent area. If the transparent area is ≤ 9 mm, it indicates that the material tested was resistant to *Candida albicans*, if the transparent area is 10-13 mm, it indicates intermediate resistant to *Candida albicans*, and if the transparent area is > 14 mm, it indicates that the testing material is sensitive to *Candida albicans*.[2,19]

The results of several studies were very different. Research conducted Grasso et al.[12] showed denture adhesive did not affect the growth of microorganisms of the oral cavity, in contrast to research conducted in 1971 showed Stafford Stafford and Russel[1] denture adhesive supports the growth of microorganisms such as *Candida albicans* and *Streptococcus mitis*.

The research results showed that there was no transparent area around the test substance (0 mm). Accordingly, it could be said that the denture adhesive tested could not inhibit the growth of *Candida albicans*, both containing sodium carboxymethylcellulose or a denture adhesive which has two active components, namely *poly (methylvinylether/maleic acid) sodium-calcium* salt mixed partial, denture adhesive base material which has antifungal properties and prophyl hydroxybenzoate which also serves as a preservative. Many factors influence the antifungal test results

which used against a microorganism, such as incubation time, solvent-fungal, seed pH, incubation temperature, type of seed and others.[20] *Candida albicans* was able to grow at a pH between 2-8 and will grow with the optimum at pH 3.8 to 5.6 with a temperature of 37°C for 24 hours.[2]

The results were in line with research conducted by Tjampakasari and Mardiastuti[20] who uses a Minimum inhibitory Concentration (MIC). She said that 60 of 71 samples of *Candida albicans* resistant to the antifungals tested. *Candida albicans* resistance can be affected by the dose and duration of antifungal administration, patients who undergo treatment with antifungal and do not obey the rules may also result in the use of antifungal resistance in *Candida albicans*, other than that the failure to handle the factors underlying the occurrence of Candida infection include the use of denture, biofilm formation, and resistance to the antibiotic resistance of *Candida albicans* causes.[20]

Biofilms consist of cells of microorganisms that are firmly attached to a surface so that at rest, do not easily separated or on the move. Biofilm is a form of cellular defense mechanism. Based on studies in vitro, they are able to avoid the attack host defenses, thereby increasing the resistance properties, as the ability to withstand the effects of a drug which is lethal to most members of the species.[21,22] This was in line with research by Jamilah[23] which stated that *Candida albicans* biofilms formed on denture have properties resistant against antifungal drugs commonly used for treatment of denture stomatitis, as amphotericin B, nystatin, fluconazole, and chlorhexidine. Resistance mechanism of resistant congenital (primary), acquired (secondary), and cross (episomal). Innate resistance is naturally found in microorganisms, resistance is obtained as a result of contact of the microorganisms with chemotheurapeutic and is usually caused by the spontaneous formation of new species with different characteristics, whereas the cross-resistance to other microorganisms episome can acquire by merging or by cell contact cell by cell incorporation.

Inhibition of microorganism growth process is not simple because of the efficiency of antimicrobial substances is affected at least by factors six. First, the size of the population, a larger population of microorganisms, the longer time needed to exterminate. Second, the composition of the population, the effectiveness of an antimicrobial substance is different for each of the microorganisms because each microorganism has different susceptibilities.[24] Active components in denture adhesive vary; these differences will produce different susceptibilities towards *Candida albicans*. Third, the higher the concentration of an antimicrobial agent results in the higher ability to exterminate microorganisms. Fourth, the duration, the longer the duration of antimicrobial substances that are exposed, the greater number of microorganisms killed. Fifth, the increase in temperature is able to enhance the work of several active components. Sixth, the environmental condition such as pH affects the growth of microorganisms.[24]Anticandida mechanism also affects the effectiveness of inhibition of growth of *Candida*. Rochani[25], mentioned four mechanisms of anticandida: interference with the cell membrane, inhibition of ergosterol biosynthesis in fungal cells, inhibition of fungal protein synthesis, and inhibition of fungal mitosis.

The results were in line with Grasso[12] study in 1945 showing the denture adhesive has no inhibitory to microorganisms. The results are also consistent with research conducted by Makihira et al.[16] That assessed the effectiveness of denture adhesive on the growth of *Candida albicans* in the detection of pH changes in germination medium. A study of six denture adhesives that has antifungal ability and sold freely showed that only two denture adhesive products have ability to suppress the growth of *Candida albicans* and one denture adhesive of the products effectively reduce the growth of Candida tropicalis.[16] Currently, there is a denture adhesive containing *poly (methylvinylether/maleic acid)-zinc mixed partial calcium salts, hexachlorophene, sodium tetraborate*, and ethanol, that reported have antimicrobial power, but more research is needed to test the effectiveness of the active components in reducing mold growth. The use of denture adhesive should remain targeted as its main function, as an ingredient that can improve denture retention and stability, so that th denture adhesive users are advised to maintain optimal oral hygiene.[26]

## CONCLUSION

Based on this research, it can be concluded that there was no antifungal activity in denture adhesive containing either *poly (methylvinylether/maleic acid) sodium-calcium* salt and mixed partial denture adhesive containing prophyl hydroxybenzoate and sodium carboxymethylcellulose towards the growth of *Candida albicans*.

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