International Journal of Clinical Science and Medical Research

ISSN(print): 2770-5803, ISSN(online): 2770-582X

Volume 05 Issue 07 July 2025

DOI: https://doi.org/10.55677/IJCSMR/V5I7-03/2025, Impact Factor: 8.005

Page No : 171-176

Effect of Soaking Heat Cured Acrylic Resin on *Citronella* Extract Solution (*Cymbopogon nardus*) on *Streptococcus mutans* Colony

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ABSTRACT **Published Online : July 08, 2025**

heat cured acrylic resin is a common material for denture bases. This material has disadvantages, such as porosity that cause plaque formation which can cause *Denture stomatitis*, such as *Streptococcus mutans*. The common preventive action was cleaning the denture bases with chlorhexidine, but it has side effects. The alternative natural disinfectant that is currently being developed as a soaking agent for heat cured acrylic resin plates, but still has limited research data is citronella. *Citronella* has antibacterial properties such as essential oils, saponins and flavonoids.

Objective: This study aims to explain the effect of immersion the heat cured acrylic resin on the number of *Streptocooccus mutans* colonies.

Methods: Invitro experimental research using a post-test with control group design. Immersion of heat cured acrylic plates in 20% citronella leaf extract solution as the treatment group and immersion in distilled water as the negative control group.

Results: The number of *Streptococcus mutans* colonies on immersion of acrylic plates in a solution of 20% citronella extract and distilled water respectively was 2.1×10^5 CFU/cm^2 and 1.4×10^6 CFU/cm^2.

Conclusion: There is an effect of soaking heat-cured acrylic resin in 20% concentration of citronella leaf extract solution on *Streptococcus mutans* colonies.

KEYWORDS:
Heat cured acrylic resin, *Citronella*, *Streptococcus mutans*.

INTRODUCTION

According to the American Dental Association, acrylic resin as denture plates can be divided into heat cured and self-cured.[1] Since the 1940s until now, heat cured acrylic resin is still the main choice as a denture plate material with a prevalence of 95%.[2] This heat cured acrylic resin was chosen because of its various advantages, such as biocompatible with oral tissue, has a color that resembles oral tissue so that it has good aesthetics, affordable price and easy to repair.[3] Among its various advantages, this heat cured acrylic resin still has disadvantages such as porosity.[2] This porosity property can make the heat cured acrylic resin absorb saliva proteins so as to form acquired denture pellicle. After the acquired denture pellicle is formed, there will be a buildup of microorganisms in the form of fungi and bacteria, such as *Candida albicans* and *Streptococcus mutans* which will form an uncalcified soft layer or can be called plaque. This plaque can cause bad breath, discoloration of dentures, periodontal problems, and denture stomatitis.[4]

Denture stomatitis is an inflammatory reaction in the mucosal area below the base of the denture. More than 50% of denture users experience denture stomatitis, and it is more common with partial dentures.[5] Denture stomatitis can cause changes in the pH of the saliva to become more acidic. This change in pH occurs due to the fermentation of carbohydrates produced by *Streptococcus mutans*. *Streptococcus mutans* is a gram-positive bacterium commonly found in the oral cavity.

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Cite this Article: Mirna Febriani, Poetry Oktanauli, Herlia Nur Istindiah, Fransiska Nuning Kusmawati, Tuti Alawiyah, Wilson Christian (2025). Effect of Soaking Heat Cured Acrylic Resin on Citronella Extract Solution (Cymbopogon nardus) on Streptococcus mutans Colony. International Journal of Clinical Science and Medical Research, 5(7), 171-176

Streptococcus mutans has both acidogenic and acidic properties.[4] The growth of *Streptococcus mutans* can be prevented by reducing carbohydrate foods, improving dental and oral health and hygiene, and using cariogenic bacterial inhibitors.[6]

Efforts that can be made to prevent denture stomatitis are cleaned at the base of dentures, one of which is by soaking using chemical disinfectants. Chemical disinfectants that are often used for dentures include sodium hypochlorite, alkaline peroxide, chlorhexidine, enzymes, acids, and castor oil. However, this chemical disinfectant has many drawbacks such as causing discoloration at the base of dentures, irritation, allergic reactions, impaired sense of taste, and can cause bacterial resistance. In addition, chemical disinfectants available on the market tend to be expensive. Herbal ingredients are considered as a substitute option for synthetic antibacterial. Herbal ingredients are believed to have fewer side effects and a more affordable price compared to synthetic antibacterial. This makes people more interested in herbal ingredients.[7,8] One of the herbal ingredients currently being developed to be used as an alternative disinfectant for dentures is citronella leaves (*Cymbopogon nardus*).

Fragrant lemongrass leaves are often found in various regions in Southeast Asia, both as a spice in cooking and as a medicine.[9] Citronella leaves contain a variety of antibacterial compounds, such as essential oils, saponins, polyphenols and flavonoids. The content of saponin compounds found in citronella leaves has been proven to be effective in inhibiting the growth of gram-positive bacteria.[10]

Dewi, et al. (2015) conducted a study on the minimum inhibitory concentration test with the test material of dried citronella plants extracted in stages with petroleum followed by 70% ethanol. The method used in the test was microdilution of biofilm formed on a flat flexible U-bottom PvC 96 wells microplate with staining with 1% *crystal violet*. The test results showed that citronella extract had an antibacterial effect on Streptococcus mutans, which was indicated by a minimum inhibitory concentration value at the level of 0.18% b/v and had a biofilm inhibition effect against *Streptococcus mutans* which was shown by an *inhibition concentration* price of 0.137%.[11]

The research of Rizkita, et al. (2017), conducted a minimum growth inhibition concentration test with citronella leaf extract at concentrations of 5%, 7%, 10%, 15%, and 20%. As a result of the study, the minimum inhibitory concentration to inhibit the growth of *Streptococcus mutans* was obtained by 20%. The study also compared the effectiveness of citronella with green betel extract (*Piper betle.* L) and red ginger extract (*Zingiber officianale var. Rubrum*) at a concentration of 20% against *Streptococcus mutans*. The results obtained were that citronella was the most effective in inhibiting *Streptococcus mutans* compared to green betel and red ginger at a concentration of 20% with an inhibitory power produced by citronella extract of 7.90 mm.[10]

Research conducted by Mayasari, et al. (2019), carried out an inhibition test using citronella leaf juice at concentrations of 10%, 20%, 30%, 40% and 50%. The results of the study found that concentrations of 20%, 30%, 40% and 50% were able to inhibit the growth of Streptococcus mutans bacteria with the average diameter of the inhibition zones formed being 14.2 mm, 15.1 mm, 16.2 mm and 17.3 mm.[12]

In the research of Hasibuan, et al. (2021), an inhibitory test of *Streptococcus mutans colonies* was carried out using citronella extract with a concentration of 20% which was compared to temulawak extract (*Curucuma xanthorrhiza*) with the same concentration. The results of the study showed that citronella extract had more optimal results than temulawak extract at the same concentration. This was obtained from the average value of the diameter of the inhibition, which was larger than that of temulawak extract, which was 12.00±0.23 mm.[13]

However, there are differences in the research conducted by Andayani (2022). This study used kitchen lemongrass leaf extract (*Cymbopogon citratus*) which has a difference in the shape of the base of the stem is larger, the color of the stem is whiter, and the aroma is not too sharp compared to fragrant lemongrass (*Cymbopogon nardus*). This study used a concentration of kitchen lemongrass extract of 20%, 40%, 60%, 80% and 100%. The results of the study found that the extract from kitchen lemongrass leaves was not effective in inhibiting the growth *of Streptoccoccus mutans* even though it had the same content as citronella.[14]

Andry's (2022) research using avocado leaves (*Persea americana mill*) has been conducted to determine the number of *Streptococcus mutans bacterial colonies* in heat-cured acrylic resin plate specimens using the pour plate *calculation method*. Avocado leaf extract in this study was not effective in inhibiting the number of *Streptococcus mutans colonies*.[15]

Citronella extract (*Cymbopogon nardus*) from various studies has been tested to have an inhibitory effect and a minimum inhibitory concentration against *Streptococcus mutans* bacteria. However, currently there are still few evaluations regarding the effect of *heat cured* acrylic resin immersion on citronella extract solution on *Streptococcus mutans colonies*, so researchers are interested in further research on the effect of *heat cured* acrylic resin immersion on citronella extract solution on *Streptococcus mutans* colonies.

MATERIALS AND METHODS

The type of research used in this study is laboratory experiments using Post-Test Only Group Design. The sample used in this study was a heat-cured acrylic resin in the form of a beam with a measurement of 10mm×10mm×2mm which was soaked in a solution of citronella extract and aquadest. There are 2 working groups with each group consisting of 16 samples, so the total sample used is 32 samples. This sample was cultured with *Streptococcus mutans* bacteria and soaked

in a solution of citronella extract and aquadest for 30 minutes and the number of colonies was tested after dilution of the flakes using the pour plate technique and using a colony counter.

RESULTS

The data obtained in this study had normally distributed data ($p<0.05$) in the Shapiro-Wilk normality test and had homogeneous data (Sig. $0.051<0.05$) in the Levene homogeneity test, so it can be said that the variation between variables in this study is relatively the same.

Table 1. Number of *Streptococcus mutans* after 30 minutes of immersion

No.	Immersion solution	Culture Form	Result	Unit
1	Citronella Extract Acrylic Resin 20%	Dense	3,6 x 105	Cfu/cm2
2			1,9 x 105	
3			3,6 x 104	
4			1,5 x 105	
5			3,2 x 105	
6			1,3 x 105	
7			2,2 x 104	
8			2,8 x 105	
9			3,3 x 105	
10			2,5 x 105	
11			2,4 x 105	
12			6,6 x 104	
13			2,9 x 105	
14			7,7 x 104	
15			2,7 x 105	
16			3,2 x 105	
1	Aquadest	Dense	1,7 x 106	Cfu/cm2
2			1,6 x 106	
3			1,8 x 106	
4			3,2 x 105	
5			9,5 x 105	
6			1,3 x 106	
7			1,4 x 106	
8			1,5 x 106	
9			1,4 x 106	
10			1,6 x 106	
11			1,4 x 106	
12			1,4 x 106	
13			7,0x 105	
14			1,4 x 106	
15			1,4 x 106	
16			2,6 x 106	

Table 2. Descriptive data on the number of *Streptococcus mutans* after soaking in 20% citronella leaf extract and aquadest

Group	N	Minimum	Maximum	Mean	Std. Deviation
Citronella Extract Acrylic Resin 20%	16	2.2×104	3.6×105	2.1×105	113399.717
Aquadest *Acrylic Resin*	16	7×105	2.6×106	1.4×106	492533.840
Valid N (listwise)	16				

Table 3. Data Normality Test

Group	Shapiro-Wilk			Interpretation
	Statistic	df	Mr.	
Citronella Extract Acrylic Resin 20%	.914	16	.136	Homogeneous
Aquadest Acrylic Resin	.889	16	.054	Homogeneous

Table 4. Data Homogeneity Test

Var.	Levene Statistic	Sig.	Conclusion
Streptococcus mutans	4.148	0.051	Homogeneous

Table 5. Independent T Test

Types of tests	Group	Average	SD	Mean Difference	Yield (Sig)
Independent t-test	Fragrant lemongrass	2.1×105	113399.717	-2.2×105	.000
	Aquadest	1.4×106	493193.252	-2.2×105	.000

The results of the independent t-test above showed that the significance value of this test was .000 with P<0.005, so it can be concluded that there was a significant difference (significantly lower) in the number of *Streptococcus mutans colonies* from acrylic resin plates soaked in a 20% citronella extract solution compared to acrylic resin plates soaked in aqueducts (negative control).

DISCUSSION

This study uses heat cured acrylic resin. According to Shen in 2021, heat cured acrylic resin is still the main choice in the manufacture of denture bases. This is based on its relatively low price, non-toxic to the oral environment, no irritation of the tissues in the mouth, good physical and aesthetic properties, and easy in the manufacturing process. However, this heat cured acrylic resin has a disadvantage in the form of its porosity properties. This porosity property makes food scraps and microorganisms easily adhere to the acrylic resin.[16]

The bacteria used in this study was *Streptococcus mutans* (ATCC 31967). *Streptococcus mutans* was chosen because it is a gram-positive bacterium that is very often found in the oral cavity. *Streptococcus mutans* on uncleaned denture plates, especially on plates attached to the surface of the oral cavity, can form plaques that can trigger *denture stomatitis.*[2,4,6]

This study used a solution of citronella extract (*Cymbopogon nardus*) at a concentration of 20% with the infusion method as a treatment and aqueous as a negative control. The preparation of this citronella leaf extract solution uses the infused method. This infusion method was chosen because it is easy to do in daily practice. The purpose of extraction is to extract compounds that have antibacterial effects. The antibacterial effects contained in citronella leaves include essential oils, saponins and flavonoids.[10]

The method of counting bacterial colonies in this study is using the pour plate technique, as the most used method of counting the number of bacterial colonies.[17] This technique is performed by diluting the sample solution and dripping it on a petri container. After that, it is flattened with a circular motion on the table slowly and incubated. The number of colonies can be calculated using a colony counter.[18]

The results (Table 5.2) showed that the heat cured acrylic resin plates soaked in citronella leaf extract solution at a concentration of 20% had fewer bacterial colonies on average than the number of bacterial colonies on the heat cured acrylic resin plates soaked in aqueducts with an average number of *Streptococcus mutans* in the soaking solution of citronella leaf extract is 2.1×105 Cfu/cm2, while for soaking in *aquadest* it is 1.4×106 Cfu/cm2. This is also supported by the significance value in the independent t-test (Table 5.5), which is .000 which shows a significant difference between acrylic resin plates soaked in a solution of citronella leaf extract and *aquadest*. The conclusion obtained was that citronella extract was more effective in reducing the number of *Streptococcus mutans colonies* compared to aquifers.

This research is in line with research conducted by Dewi, et al. (2015), which stated that fragrant lemongrass leaf extract has an antibacterial effect and can inhibit the formation of biofilms. This is based on the presence of essential oils, saponins and flavonoids that are effective in inhibiting the growth of *Streptococcus mutans.*[11]

This research is also in line with the research conducted by Rizkita, et al. (2017), using concentrations of 10%, 20%, 40%, 60% and 80%. The results obtained are that at a concentration of 20%, citronella extract can inhibit the

growth of *Streptococcus mutans* and is more effective compared to green betel extract (*Piper betle L.*) and red ginger (*Zingiber officianale var. Rubrum*) at a concentration of 20%. This study also states that the ingredient that makes citronella more effective in inhibiting the growth of *Streptoccoccus mutans* is the tripterpenoids in essential oils, namely geraniol is a direct derivative of alcohol.[12]

This research is directly proportional to the research conducted by Mayasari, et al. (2019), which stated that at concentrations of 20%, 30%, 40% and 50%, the juice of citronella leaves can inhibit the growth of *Streptococcus mutans* bacteria. This study states that the content of flavonoids in citronella affects the antibacterial effect. In addition to flavonoids, tannins also contribute to disrupting the permeability of the bacterial wall.[13]

This research is also supported by research conducted by Hasibuan, et al. (2021), which stated that at a concentration of 20%, citronella extract is more effective in inhibiting the growth of *Streptococcus mutans* compared to temulawak extract (*Curcuma xanthorrhiza*) at a concentration of 20%. This is based on the complex content of essential oils, saponins and flavonoids contained in citronella.[14]

However, this study is not in line with the research conducted by Andayani (2022). In this study, kitchen lemongrass extract (*Cymbopogon citratus*) was used with concentrations of 20%, 40%, 60%, 80% and 100%, the result was that this kitchen lemongrass was not effective in inhibiting the growth of *Streptococcus mutans.* This kitchen lemongrass contains flavonoids and tannins which are also contained in citronella.[15]

According to Bota (2015), essential oil from citronella extract has three main ingredients, namely citronella, citronellol and geraniol. The antibacterial mechanism is to destroy the cell wall and damage the intracellular tissue in bacteria. These three main ingredients show a strong antibacterial effect on gram-positive bacteria. *Streptococcus mutans* is a gram-positive bacterium so the content of essential oils in citronella is effective in inhibiting the growth of *Streptococcus* mutans.[19]

According to Anggraini (2019), saponins are antibacterial that work by damaging the permeability of cell walls, resulting in cell death. Saponins have been shown to be effective in inhibiting the growth of gram-positive bacteria.[20]

According to Manik (2017), flavonoids can also inhibit the growth of *Streptococcus mutans*. The mechanism of action of flavonoids is to release transduction energy in the cytoplasm and inhibit the individual movement of bacteria, and the hydroxyl group of these flavonoids can have a toxic effect on bacteria by inhibiting protein transport and changing the organic components of a bacterium.[21]

According to Saptowo (2022), the mechanism of action of tannins as antibacterial is to cause sellisis. Tannins make polypeptide walls as targets that can cause cell wall formation to be less than perfect and result in death in bacterial cells. In addition, tannins can also inactivate enzymes from bacteria so that protein transport is disrupted.[22]

Based on the above research, it has been proven that a solution of citronella leaf extract at a concentration of 20% can affect the growth of the number of *Streptococcus mutans* colonies on heat-cured *acrylic resin plates* and is more effective in inhibiting the growth of *Streptococcus mutans colonies* compared to aquadesttics. This is based on the presence of antibacterial compounds such as saponins, flavonoids, tannins and essential oils in citronella which are effective in inhibiting the growth of *Streptococcus mutans* and the absence of antibacterial compounds contained in aquadesttics so that citronella extract at a concentration of 20% can be used as a substitute for chemical disinfectants in inhibiting the growth of *Streptococcus mutans*.

CONCLUSIONS

From the results obtained in this study, regarding the effect of soaking of heat cured acrylic resin on the solution of citronella extract (*Cymbopogon nardus*) on *Streptococcus mutans colonies*, it was found that the solution of citronella leaf extract (*Cymbopogon nardus*) 20% had an effect on the immersion of heat cured acrylic resin plates on the number of *Streptococcus mutans* colonies and there was a significant decrease in the number of *Streptococcus mutans* colonies on heat cured acrylic resin plates soaked in a 20% solution of citronella leaf extract compared to immersion in aquifers.

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