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The Effectiveness Of Corn Silk *Zea Mays Saccharata* Extraction As The Obstruction Of *Staphylococcus Aureus* Bacteria On Acrylic Plate

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ABSTRACT

Background: Staphylococcus aureus is one of the bacteria that contribute to plaque formation and is often found on the surface of acrylic plates on removable partial dentures (RPDs). RPD cleaning can be done mechanically, chemically, or in combination. Sweet corn silk (Corn silk Zea mays Saccharata) can be used as an alternative natural ingredient for RPD cleaners because Corn silk Zea mays Saccharata contains flavonoids, saponins, and alkaloids that can act as antibacterials. Purpose: This study aims to determine the effect of Corn silk Zea mays Saccharata extract in inhibiting the growth of Staphylococcus aureus bacteria on acrylic plates. Materials and Methods: This study used sweet corn silk with laboratory experimental research type using a post-test only control group design approach. The maceration method using 70% ethanol solvent was carried out to make Corn silk Zea mays Saccharata extract and then diluted to obtain concentrations of 25%, 50%, and 75%. This study used 0.5% Sodium Hypochlorite as the positive control and aquades as the negative control. Results: The lowest average growth of Staphylococcus aureus bacteria on acrylic plates was when Corn silk Zea mays Saccharata was given at 75%, which was 366.40 CFU/ml, but not as low as Sodium Hypochlorite, which was 0 CFU/ml. While the highest average growth of Staphylococcus aureus bacteria on acrylic plates was the negative control at 1196.80 CFU/ml. Conclusion: Corn silk Zea mays Saccharata extract using ethanol solvent can inhibit the growth of Staphylococcus aureus bacteria on acrylic plates with 75% as the concentration that has the highest level of effectiveness.

Keywords: Staphylococcus aureus, RPD, Corn silk Zea mays Saccharata.

INTRODUCTION

Acrylic resin is a material used in making removable partial dentures (RPDs) and is one of the dental instruments in the field of prosthodontics that is made with the aim of replacing missing teeth, protecting supporting tissue, and able to restore chewing function, speech function, and improve aesthetics. The

lack of patient awareness in maintaining oral hygiene and cleanliness of the used RPD will cause plaque accumulation.1,2 Research conducted by Pereira et al (2013) stated that Staphylococcus aureus is microorganism most frequently found on the surface of acrylic plates and is a bacterium that plays an important role in plaque formation with a significantly higher number compared to other Staphylococcus bacterial species in patients that are wearing RPD.3.4

Cleanliness of RPD is very much needed in patients with RPD. Research by Sofya et al (2016) explains that cleaning RPD itself can be done in several ways, including mechanically, chemically or combined.5 Sodium hypochlorite (NaOCl) is one of the materials often used in cleaning RPD. According to Moreira et al (2015), the concentration of 0.5% in NaOCl is the most effective in controlling biofilm formation and inhibiting the growth of microorganisms on RPD.6 However, in the research conducted by Arruda et al (2015), it was explained that the use of 0.5% NaOCl over a long period of time can cause color changes in acrylic-based RPD.7

Cleaning RPD in addition to chemical methods, can be done using natural ingredients, one of the ingredients that has not been optimally utilized is sweet corn silk (Corn silk Zea mays Saccharata). Sweet corn silk (Corn silk Zea mays Saccharata) is often found in Indonesia, especially in South Sumatra where the community does not yet know the benefits and content in inhibiting bacterial growth. Corn silk Zea mays Saccharata contains tannin, carbohydrates, beta-carotene, fatty oils, allantoin, bitter substances and flavonoids, steroids, alkaloids, and saponins which play an important role in inhibiting antibacterial activity.8–10 This study was conducted using Corn silk Zea mays Saccharata harvested at the ripe phase based on the research of Sarepoua et al (2015) which explains that Corn silk Zea mays Saccharata produces good quality at the silking phase (milk ripeness) with a high amount of flavonoid content and antioxidant activity compared to the dought phase (physiological ripeness).9

Research on the antibacterial activity and phytochemical properties of Corn silk extract Zea mays Saccharata have previously been conducted. Selim et al (2015) in their research used Corn silk Zea mays Saccharata extract with 3 types of solvents, namely ethanol, methanol, and chloroform with the results showing

that Corn silk Zea mays Saccharata extract using ethanol solvent gave good performance results in antibacterial activity with the highest inhibition zone on Staphylococcus aureus bacteria compared to methanol and chloroform solvents.10 Research on Corn silk Zea mays Saccharata extract was also conducted by Haslina and Sri Untari (2017) using methanol solvent with concentrations of 25%, 50%, and 75% which showed that 75% with an extraction time of 50 minutes had the largest average diameter of the inhibition zone for Staphylococcus aureus bacteria compared to concentrations of 50% and 25%.11

Therefore, this study will be conducted using ethanol solvent on Corn silk Zea mays Saccharata extract with concentrations of 25%, 50%, and 75% with the aim of seeing whether these concentrations using different solvents, namely ethanol, can also inhibit the growth of Staphylococcus aureus bacteria on acrylic plates.

RESEARCH METHODS

The research was conducted in August 2022 at the Chemistry Laboratory of Politeknik Negeri Sriwijaya for the manufacture of Corn silk Zea mays Saccharata extract and Balai Besar Kesehatan Palembang for the calculation of Staphylococcus aureus bacteria. Corn silk Zea mays Saccharata was rinsed with running water, drained, dried, then ground using an electric blender to obtain 500g of dry Corn silk Zea mays Saccharata powder. The powder is soaked in 70% ethanol solvent through a maceration process for 3x24 hours and stirred occasionally then filtered. The filtrate from the maceration that has been obtained is concentrated using a vacuum rotary evaporator at a temperature of 600C until a concentrated extract is obtained. Furthermore, the concentrated extract is diluted with distilled water to obtain Corn silk Zea mays Saccharata extract with concentrations of 25%, 50%, and 75% using the formula M1.V1 = M2.V2.12,13,14,15

Sodium Hypochlorite (NaOCl) is a chemical solution for cleaning dentures that will be used as a positive control in this study with the concentration of 0.5%. Staphylococcus aureus bacterial colonies were placed in a test tube containing Nutrient Agar (NA) media at the position of 15 which has been sterilized for 15

minutes using an autoclave at the temperature of 121C. Inoculate Staphylococcus aureus bacteria into Nutrient Agar (NA) media and incubate in an incubator for 24 hours at the temperature of 37C.

The research sample used in this study was a 10x10x1 mm (n=25) acrylic plate with the criteria of no macro pores, flat plate surface, and smooth. The acrylic plate was sterilized using an autoclave at a temperature of 121oC for 1 hour. After sterilization, the acrylic plate was inserted into sterile artificial saliva for approximately 1 hour with the aim that the condition of the acrylic plate was the same as the conditions in the oral cavity. Furthermore, the acrylic plate was contaminated with Staphylococcus aureus bacteria by inserting the acrylic plate into a test tube containing a suspension of Staphylococcus aureus bacteria and incubated for 24 hours at the temperature of 37oC.

The samples were divided into 5 groups, each group consisting of 5 test tubes containing Corn silk Zea mays Saccharata extract at concentrations of 25%, 50%, 75%, 0.5% Sodium Hypochlorite as the positive control, and distilled water as the negative control with a soaking time of 30 minutes. Furthermore, the acrylic plate was removed and rinsed with phosphate buffer saline 2 times to clean the remaining soaking solution.

After rinsing, the acrylic plate was soaked in 10 ml of 0.9% NaCl in a test tube and vibrated with a vortex for 30 seconds to release the remaining Staphylococcus aureus bacteria. Then, take 0.1 ml of Staphylococcus aureus bacterial suspension using a syringe from the solution and drop it on the Nutrient Agar (NA) media in a petri dish, incubated for 2x24 hours at 37oC and the number of Staphylococcus aureus bacterial colonies was calculated in each group using a Colony counter. Data processing and analysis were done by statistical tests.

RESULTS AND DISCUSSION

Results

This study is a laboratory experimental study using a post-test only control group design approach which aims to determine the effectiveness differences of Corn silk *Zea mays Saccharata* extract with ethanol solvent in various concentrations on the growth of *Staphylococcus aureus* bacteria on acrylic plates. This study was

conducted to analyze the characteristics of the research data on the growth of *Staphylococcus aureus* bacteria on acrylic plates with each concentration of Corn silk *Zea mays Saccharata* extract with ethanol solvent which can be seen based on the average, minimum, maximum and standard deviation values shown in table 1.

Table 1. Growth of Staphylococcus aureus Bacteria on Acrylic Plates

Corn silk Zea mays Saccharata extract	Average	Min	Max	Standard Deviation
75%	366.40	226	511	104.99
50%	668.80	436	1024	232.43
25%	887.40	569	1219	248.67
Control (-)	1196.80	970	1459	211.61
Control (+)	0.00	0	0	0

Based on Table 1. the results of this study indicate that the average growth of *Staphylococcus aureus* bacteria on the acrylic plate is lowest when Corn silk *Zea mays Saccharata* is given using 70% ethanol solvent with a concentration of 75% with a value shown of 366.40 CFU / ml, while the average growth of *Staphylococcus aureus* bacteria on the acrylic plate is highest is the negative control, which is distilled water that has an average value of 1196.80 CFU / ml and Corn *silk Zea mays Saccharata* with ethanol solvent at the concentration of 25% which has an average value of 887.40 CFU / ml. The positive control treatment has an average growth of *Staphylococcus aureus* bacteria at 0 CFU / ml which explains that there is no growth of Staphylococcus aureus bacteria.

Results of the Normality Test of *Staphylococcus aureus* Bacterial Growth on Acrylic Plates

The normality test of the growth data of *Staphylococcus aureus* bacteria on acrylic plates based on 5 treatments will be carried out to determine whether the research data has been normally distributed or not. In this study, the Kolmogorov Smirnov test was used to measure the normality of the data before the One Way Anova test was carried out. This study uses a significance level of 5%, so that if the

p value is obtained > 0.05 meaning that the research data has been normally distributed. The following are the results of the normality test in this study:

Table 2. Results of Normality Test (Kolmogorov Smirnov) of *Staphylococcus aureus* Bacterial Growth

Concentration Corn silk *Zea mays Saccharata*	*p*	Distribution
75%	0,200	Normal
50%	0,200	Normal
25%	0,200	Normal
Control (-)	0,200	Normal
Control (+)	0,000	Abnormal

p> 0,05 (Normally distributed)

Table 2. shows the results of the normality test of *Staphylococcus Aureus* bacterial growth on acrylic plates of 25%, 50%, 75% concentration groups of Corn silk *Zea mays Saccharata* extract with ethanol solvent and negative control have a p value > 0,05 so that the data has been normally distributed. While the positive control has p < 0,05 which means the data is not normally distributed. Because this study found data that was not normally distributed, it could not fulfill the use of the One Way Anova test.

Homogeneity Test of *Staphylococcus aureus* Bacterial Growth on Acrylic Plates

The homogeneity test in this study was analyzed using the Levene test to determine whether each treatment group has a homogeneous (same) or heterogeneous (different) variance. The homogeneity test is one of the requirements before conducting the One Way Anova test. If each treatment group has a homogeneous variation, the research results will be more accurate when a different test is carried out. At a significant level of 5%, if the p value > 0,05 means that the research data has homogeneous variance. The following are the results of

the homogeneity test of *Staphylococcus aureus* bacterial growth at each concentration.

Table 3. Results of Homogeneity Test (Levene test) of Staphylococcus aureus Bacterial Growth

Concentration *Corn silk Zea mays Saccharata*	***p***	**Information**
75%		
50%		
25%	0,011	Not Homogeneous
Control (-)		
Control (+)		

p> 0,05 (Homogeneous Variance)

Table 3 shows the results of the homogeneity test of *Staphylococcus aureus* bacterial growth on acrylic plates in each group of Corn silk *Zea mays Saccharata* extract 25%, 50%, 75% with ethanol solvent, negative control and positive control have p (0.011) < 0.05 which means that the research data has a non-homogeneous variance. The results of the normality test and homogeneity test indicate that the growth data of Staphylococcus aureus bacteria have not met normality and homogeneity so that a one-way anova test cannot be carried out in measuring the effectiveness of Corn silk *Zea mays Saccharata* extract with ethanol solvent. The test that can be used is the krusskall wallis test, followed by the post hoc test, namely mann whitney.

Kruskal Wallis Difference Test of Bacteria *Staphylococcus aureus*

The Kruskall Wallis statistical test was conducted to determine whether there was a difference in the number of *Staphylococcus aureus* bacterial colonies in each treatment group when the data was not normally distributed and was not homogeneous. This study used a significance level of 5% if the p value < 0.05 means there is a significant difference between the concentration of Corn silk *Zea*

mays Saccharata extract. The following are the results of the Kruskall Wallis test on the growth of the number of *Staphylococcus aureus* bacterial colonies:

Table 4. Kruskall Wallis Test Results for *Staphylococcus aureus* Bacterial Growth

Concentration *Corn silk Zea mays Saccharata*	**Mean + SD**	***p***	**Information**
75%	366, 40 ± 104,99	0,000	Significantly Different
50%	668,80 ± 232,43		
25%	887,40 ± 248,67		
Control (-)	1196,80 ± 211,61		
Control (+)	0,00 ± 0,00		

Information: SD = Standard Deviation

In the Table 4. shows the results of the Kruskall Wallis test of the growth of *Staphylococcus aureus* bacteria on acrylic plates at each concentration of Corn silk *Zea mays Saccharata* extract with ethanol solvent has a p value (0.000) < 0.05, it can be concluded that there is a significant difference in the number of *Staphylococcus aureus* bacterial growth at each concentration. Therefore, the next stage is to conduct a Post Hoc test using the Mann Whitney test to determine the difference in the growth of the number of colonies in each treatment group

Table 5 shows the results of the Mann Whitney test for the administration of a concentration of 75% *Zea mays Saccharata* Corn silk extract with ethanol solvent which is significantly different from the concentrations of 50%, 25%, negative control and positive control ($p < 0.05$).

Table 5. Post-Hoc Test Results (Mann Whitney) of *Staphylococcus aureus* Bacterial Growth

Group	75%	50%	25%	Negative Control	Positive Control
75%					
50%	0,016*				
25%	0,009*	0,175			
Control (-)	0,009*	0,028*	0,047*		
Control (+)	0,005*	0,005*	0,005*	0,005*	

Description: Sign (*) = Significantly different at a significance level of 5% ($p < 0.05$)

Overall, it can be concluded that the concentration of Corn silk *Zea mays Saccharata* extract of 75% with ethanol solvent as the highest level of effectiveness in inhibiting the growth of *Staphylococcus aureus* bacteria. Thus, a concentration of 75% is the best concentration in inhibiting bacterial growth of *Staphylococcus aureus.*

In the second order that can inhibit the growth of *Staphylococcus aureus* bacteria is 50% and 25% concentration. The effectiveness levels of 50% and 25% concentrations have results that are not significantly different from each other, resulting in almost the same bacterial growth, although in terms of average concentration 50% has a lower value. In terms of Mann Whitney testing, both have almost the same results.

Discussion

*Staphylococcus aureus*is one of the normal flora found in the oral cavity. The number of normal flora in the oral cavity in each individual is different, because each individual has a different body defense system. In addition, the composition of normal flora can also be influenced by several factors such as diet, antibiotic use, and host. [15] The results of this study can be seen in table 1. shows that the concentration of 75% Corn silk *Zea mays Saccharata* extract has an average number of *Staphylococcus aureus* bacterial growth that is not as large as

the positive control, namely 0.5% *Sodium Hypochlorite* with an average result of 0 CFU / ml, with the explanation that there is no growth of *Staphylococcus aureus* bacteria. This is because 0.5% *Sodium Hypochlorite* can inhibit the growth of microorganisms and control the formation of biofilms. [16] Research by Tiara et al. (2014) explains that one of the normal flora, namely *Staphylococcus aureus*, has an important role in the body's defense because this normal flora produces a substance to inhibit the growth of other microorganisms.[17] So it can be concluded that if there are no *Staphylococcus aureus* bacteria as normal flora in the oral cavity, then the body's defenses which play a role in inhibiting the growth of other microorganisms will also be disrupted.

Overall, the average number of *Staphylococcus aureus* bacterial growth on acrylic plates using Corn silk *Zea mays Saccharata* extract with ethanol solvent at a concentration of 75% has higher level of effectiveness compared to concentrations of 25% and 50%. Corn silk *Zea mays Saccharata* extract with its ability to inhibit *Staphylococcus aureus* bacteria can be caused by flavonoids, saponins, and alkaloids contained in Corn silk *Zea mays Saccharata* which act as antibacterials. Flavonoids have a working mechanism, namely by damaging the permeability of bacterial cell walls, microsomes, and lysosomes which are the result of the interaction between bacterial DNA and flavonoids.

Flavonoid content can also damage bacterial cell membranes by forming a complex compound of dissolved extracellular proteins. Saponins as one of the antibacterial contents of Corn silk *Zea mays Saccharata* working mechanism is by disrupting and reducing the stability of the cell membrane by diffusing through the cell wall and outer membrane then binding to the cytoplasmic membrane causing the cytoplasm to leak and exit the cell which can cause cell death. The mechanism of action of alkaloids is by damaging the synthesis of peptidoglycan in bacterial cells so that the formation of imperfect or intact cells occurs. This can cause death in these cells because the cells do not contain peptidoglycan.[18.19]

This study is in line with the study by Haslina and Sri Utari (2017) which had similar results using a different solvent, namely methanol, and the results shows that the higher the concentration used in the Corn silk *Zea mays*

Saccharata extract, the more effective it is in inhibiting the growth of *Staphylococcus aureus* bacteria on acrylic plates.11

This research was conducted using ethanol solvent, this is based on research by Asrori M et al. (2020) explaining that methanol has toxic properties if swallowed, inhaled, and comes into contact with the skin and is flammable. [20] In contrast to ethanol, based on research by Jusuf M (2010), ethanol is a form of alcohol that is often found in alcoholic beverages such as wine, beer, whiskey and has also long been used as a medicine with the property of causing a burning sensation in the throat if swallowed.[21]

So it can be concluded that in this study, Corn silk *Zea mays Saccharata* extract with ethanol solvent used as a natural ingredient of RPD cleaner is able to inhibit the growth of *Staphylococcus aureus* bacteria on acrylic plates with the most effective concentration being 75%. Further research that may be done is to see other abilities possessed by Corn silk *Zea mays Saccharata* extract with ethanol solvent besides as an antibacterial of a RPD cleaner.

CONCLUSION

Based on the research results, it can be concluded that the extract of Corn silk *Zea mays Saccharata* using ethanol solvent can inhibit the growth of *Staphylococcus aureus* bacteria on acrylic plates with 75% as the concentration that has the highest level of effectiveness in inhibiting the growth of *Staphylococcus aureus* bacteria on acrylic plates.

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