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The Spray Effect of *Peronema Canescens Jack* As a Disinfectant Against the Growth of *Staphylococcus aureus* on the Surface of Alginate Molds

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Abstract:- The jaw impression has the potential for cross-infection from blood and saliva attached to the irreversible hydrocolloid (alginate) mold so that infection control is carried out by washing with running water and disinfecting. Disinfection of alginate molds with the spray can use chemical and natural materials. *Peronema canescens jack* (*P. canescens jack*) **is found in Indonesia, one in West Sumatra, and has antibacterial properties. The sample is tubular with a diameter of 8 mm and a height of 15 mm, consisting of 6 groups, namely the concentration group of 0.5%, 1%, and 2%, as well as the positive control group and negative control group. Microbial test by examining the growth of *Staphylococcus aureus* (*S. aureus*) after being sprayed and stored for 10 minutes and then analyzed after 24 hours of incubation with a spectrophotometer. The analysis used the Kruskal-Wallis test and continued with the Mann-Whitney test. There is an effect of spraying *Peronema canescence jack* at a concentration of 0.5%, which has a more significant growth inhibition than a concentration of 1% and 2%. Disinfection using *P. canescens jack* can inhibit the growth of *S. aureus* at a 0.5%**

Keywords:- *Alginate, Disinfectant, Peronema Canescens Jack, Staphylococcus Aureus.*

I. INTRODUCTION

Artificial teeth can cause loss of teeth in prosthodontics. The success of dentures is very dependent on the impression stage, one of which is anatomical imprinting, where accurate impressions produce dentures with good adaptation. The variable that affects the jaw impression results is the impression material used by the operator (Yuzbasioglu et al., 2014).

Anatomical impressions were performed using irreversible hydrocolloid impression material (alginate). The results of this printout will be used to create a study model and work model to support the determination of the treatment plan. The advantage of this printing material is that it is easier to manipulate, does not require a lot of equipment, is relatively inexpensive, is comfortable when applied to patients, and the printouts are accurate (Onwubu and Okonkwo, 2021). The disadvantage of this printing material is that it has imbibition properties. Namely, it absorbs water when contaminated with a wet environment so that it expands quickly and shrinks easily due to the release of water content (syneresis) (Anusavice and Phillips, 2003).

The mold can be easily contaminated with saliva, plaque, blood, and pathogenic microorganisms during the molding process. Saliva found 50,000 potentially pathogenic bacteria in one drop of saliva (Ohara-Nemoto et al., 2008). According to Wirayuni's research (2020), it was found that 67% of alginate prints sent by dentists to the laboratory were contaminated by pathogenic bacteria, cross-infection from blood residue, or debris still attached to the surface of the molds and triggers for disease transmission. The most common pathogenic bacteria found on the surface of alginate molds were *S. aureus* (Wirayuni and Juniawati, 2020).

*Staphylococcus aureu*s often causes infection in denture stomatitis and oral mucositis, which is a risk factor for atherosclerosis, oral Chorn's disease to a high risk of infectious diseases such as hepatitis, HIV-AIDS, and tuberculosis (Wirayuni and Juniawati, 2020). The American Dental Association (ADA) recommends that impression materials be washed with water to remove saliva and blood adhering to the impressions and disinfected to avoid bacterial contamination for 10 minutes before being sent to the laboratory. Disinfection techniques that can be used are immersion and spraying techniques. The spraying technique is considered an effective method to reduce the occurrence of imbibition in the mold compared to immersion (Badrian et al., 2012).

One of the traditional medicines that grow in Indonesia and are widely used is the *P. canescens jack* which has been proven effective in preventing bacteria (Ibrahim and Kuncoro, 2012). Based on research conducted by Latief (2021) that the phytochemical test of the methanol extract of *P. canescens jack* contains the highest class of active antibacterial compounds, namely alkaloids, flavonoids, and tannins and terpenoids, other antibacterial content in the form of glycosides, steroids, and phenolics (Latief, 2021).

II. MATERIAL AND METHODS

A. *Peronema canescens jack Extraction*

Peronema canescens jack extraction was macerated in 80% ethanol for 24 hours and stirred every 4 hours. After that, it was decanted and filtered, and the residue was reprocessed in the same way for 48 hours. The filtrate was evaporated with a rotary vacuum evaporator until a solid extract was obtained. Dilution of the thick extract with PBS (Phosphate Buffered Saline) solution (1 mg: 10 mg) then vibrated as much as 25 ml to obtain concentrations of 0.5%, 1%, and 2%5.

B. *Prepare alginate molds*

Manipulation of alginate (Aroma Fine Plus®) with a ratio of powder and water 2.1: 5. Manipulate the alginate in a figure-eight motion and press it against the wall of the rubber bowl with intermittent rotation (180^0) using a spatula until homogeneous. Put the alginate material into the plastic mold and wait for it to harden, then remove the alginate from the mold (Zhou et al., 2019).

C. *Inhibition of Staphylococcus aureus assay*

Staphylococcus aureus was cultured on NB media for 48 hours. Then it was equalized with 0.5 MC Farland (1.5×10^{-8}) 2. Then the sample was immersed in 50 L of *S. aureus* solution and adapted at 35°C for 60 min. Disinfect the molds with the control group and the treatment group (0.5%, 1%, and 2%) with a spray technique, starting with spraying all parts of the alginate in a sterile atmosphere of as much as 2 ml with a spray distance of 5 cm from the sample. It was then wrapped in a tissue that had been moistened with a disinfectant solution and wrapped in sealed plastic for 10 min. Then the alginate sample was put in a centrifuge tube containing 1.5 mL of PBS solution and homogenized. The effect of the extract in inhibiting the growth of *S. aureus* was examined 24 h after incubation in the incubator11. Examination of the development of *S. aureus* based on turbidity using a spectrophotometer at a wavelength of 600 nm (Syafriza et al., 2020).

D. *Statistical Analyses*

The Data on the effect of *P. canescens jack* from different concentrations on the growth of *S. aureus* was analyzed by Mann-Whitney with a limit of the significance of $p<0.005$

III. RESULTS AND DISCUSSION

The study's results were that 25 samples were tested univariate so that each group's standard deviation and mean were obtained. Staphylococcus aureus growth using Mc Farland control with 1.5×10^8 CFU/ml. The results presented in Table 1 and Fig 1 show that the average growth of *S. aureus* after spraying *P. canescens jack*, the highest inhibition of *S.aureus* growth was found in the positive control group with an average of 0.04 nm, in the spraying group with 0.5%, the highest inhibitory power was 0.08 nm. The lowest was at a concentration of 2%, namely 0.17 nm, and had similarities with the negative control, namely 0.15 nm, indicating differences in the growth inhibition of *S. aureus*. Based on the results of the Shapiro-Wilk test, the data results are not normally distributed, so the Kruskal-Wallis test is carried out to see a significant effect on all treatment groups. It is shown in Table 2.

P. canescens jack	**Repetition**					**SDV ± Mean**
	1	**2**	**3**	**4**	**5**	
0,5%	0,076	0,078	0,078	0,077	0,087	0,004 ± 0,08
1%	0,091	0,097	0,094	0,091	0,095	0,003 ± 0,09
2%	0,167	0,169	0,169	0,168	0,166	0,001 ± 0,17
Control +	0,04	0,04	0,046	0,041	0,043	0,003 ± 0,04
Control -	0,145	0,147	0,151	0,152	0,151	0,003 ± 0,15
S. aureus	0,12	0,1	0,09	0,081	0,088	0,015 ± 0,10

Table 1:- Average growth inhibition of *S. aureus*

In Table 2. it is shown that the method of spraying *P. canescens jack* on the alginate surface can inhibit the growth of S.aureus. The value of the growth inhibition of *S.aureus* in each group showed a significant difference between the control group and the treatment group with a $p < 0.05$ ($p = 0.000$). The growth tolerance of *S. aureus* is shown that a concentration of 0.5% can tolerate the growth of Staphylococcus aureus by 15%. A concentration of 2% can provide a growing tolerance of 32%, meaning that a concentration of 0.5% has effectively inhibited bacterial growth. In comparison, a concentration of 2% has a less inhibitory effect on the growth of *S. aureus*. It can be concluded that the impact of *P. canescens jack* spray on the alginate surface shows that a concentration of 0.5% has a better inhibitory ability than concentrations of 1% and 2%.

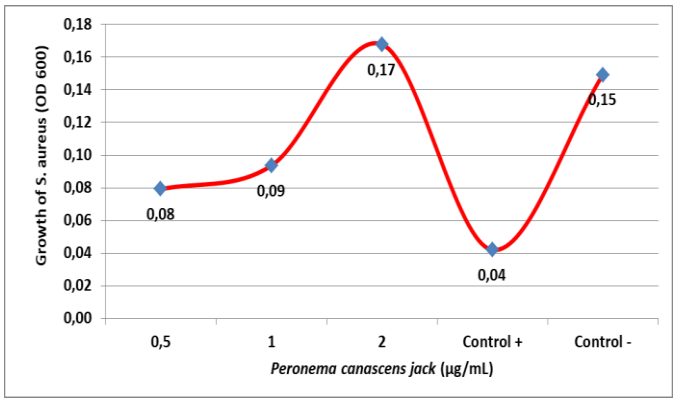

Fig 1:- Growth inhibition of *S. aureus* in the effect of spraying *P. canescens jack* extract on the surface of alginate molds

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The difference in the effect of assay material 0.5%, 1%, 2%, positive control, and negative control on alginate molds against Staphylococcus aureus was known by looking at which treatment pairs were significant between each group using the Mann-Whitney test.

The results in Table 1 and Table 2 show the value of the most significant inhibition of growth of *S.aureus* bacteria in the positive control and treatment groups with a concentration of 0.5%. Based on the results in Fig1, it can be seen that the concentration of 0.5% is better than the high concentration. The antibacterial compound content of 0.5% extract was more effective in inhibiting the growth of Staphylococcus aureus bacteria compared to 1% and 2% extract, respectively. It is because the less the concentration of the solution, the more dilute the solution will be. It causes more absorption in the alginate mold compared to high/concentrated concentrations5. These results align with the Gupta (2011) examination, which showed that concentration could affect the effectiveness of antibacterials. Still, a rise in effect does not always accompany an increase in engagement (Gupta et al., 2001). According to Lee (2005), the diameter of the bacterial inhibition zone does not always increase in proportion to the increase in antibacterial concentration. It can happen because the difference in the rate of diffusion of antibacterial compounds in the media and the concentration of different antibacterial compounds also gives a different diameter of the inhibition zone at a certain length of time (Lee et al., 2005).

P. canescens jack	**N**	**SDV**	**Mean**	**Freq**	**CFU/mL**	**MC. Farland**	**p-value**
0,5	5	0,004	0,08	15%	<300	0,5	p<0.05:0.000
1	5	0,003	0,09	18%	<300	0,5	
2	5	0,001	0,17	32%	>600	1	
Control +	5	0,003	0,04	8%	<300	0,5	
Controls -	5	0,003	0,15	28%	>600	1	

Table 2:- Distribution and frequency of *S. aureus* inhibition

The increase in the tolerance value of bacterial growth at a concentration of 2% did not ultimately occur due to bacterial growth. Still, it could also be influenced by the engagement that occurred at higher concentrations so that it could affect light absorption by dead bacterial cells (Lee et al., 2005). The number of Staphylococcus aureus in the positive control group by spraying with 0.5% sodium hypochlorite solution resulted in the lowest frequency of bacterial growth. According to the results of this study, supported by research by Gopal et al. (2015), the spray alginate mold disinfection within 10 minutes inhibited 98.5% of Staphylococcus aureus bacteria (Galie et al., 2018). Hamid et al. (2015) also reported that spraying 0.5% sodium hypochlorite on alginate mold and stored in sealed plastic for 10 minutes inhibited 98.2% of Staphylococcus aureus (Badrian et al., 2015).

The different abilities of *P. canescens jack*, can be caused by differences in the concentrations, which affect the disinfection work of microorganisms. The power of *P. canescens jack* to inhibit the growth of *S. aureus* is due to the content of secondary metabolites that have antibacterial activity, namely flavonoids, alkaloids, tannins, and terpenoids (Latief, 2021). Flavonoid antibacterial compounds function as antibacterials by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes and are phenolic compounds that are protein coagulants. The terpenoid content works by damaging the cell wall and causing the cell contents to come out (lysis) and the bacteria to die. Alkaloid compounds work by interfering with the peptidoglycan constituent components of bacterial cells so that the formation of cell walls is not intact and bacteria die (Bouyahya et al., 2022).

Lipophilic polyphenolic compounds that are lipophilic can penetrate bacterial cell walls more easily through interactions with proteins and the peptidoglycan layer because this layer is non-polar so that it can damage bacterial cell membranes, precipitate proteins through reactions with cell membranes, inactivation of enzymes, and the destruction of the function of genetic material bacteria died (Mazaya et al., 2020). The positive control group used sodium hypochlorite with a significant value with the highest inhibitory power. It happened because the sodium hypochlorite content, namely hypochlorous acid and hypochlorite ions, spread through the microbial cell membrane. Ionization of hypochlorite ions causes rupture or disintegration of microbial cell walls and membranes, and then hypochlorite ions will inactivate functional proteins. The hypochlorite ion only works outside the cell. Hypochlorous acid can penetrate the lipid bilayer of bacterial cells and attack bacterial cells from the inside to cause the rate of bacterial inactivation (Ersoy et al., 2019).

IV. CONCLUSION

Spraying of *P.canescens jack* as a disinfectant on alginate molds can inhibit the growth of *S. aureus* with an adequate concentration of 0.5%.

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