

Inhibition Potency of Allicin in the Tooth Paste Against *Lactobacillus Acidophilus*: In Vitro Study

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Received: 08.04.20, Revised: 22.05.20, Accepted: 13.06.20

ABSTRACT

Background: The allicin compound is produced after the intact *Allium sativum* tissue is destroyed or extracted. Antibacterial activity of allicin against *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Pseudomonas aeruginosa* and *Lactobacillus* spp. at concentrations of 5%, 10%, 20%, and 100%.

Purpose: the researchers are interested in conducting research on the inhibition of allicin in toothpaste on *Lactobacillus Acidophilus*.

Methods: The research conducted was an experimental laboratory study with a post-test only control group design. In this study, allicin added to toothpaste was divided into 4 groups. The data was analyzed using Anova Test and HSD.

Results. The results of the Tukey HSD test data show a significant difference if the probability value is equal to 0.05 ($p < 0.05$).

Conclusion. *Lactobacillus Acidophilus* is better inhibited by allicin in toothpaste with a higher concentration.

Keywords: *Lactobacillus Acidophilus*, allicin, *Allium Sativum*

INTRODUCTION

The oral cavity is an important part of the body for humans. The oral cavity which is not maintained in health will cause various diseases^[1]. Dental caries are one of the most common and preventable cases. Humans are susceptible to dental caries throughout their lifetime. Dental caries are a major cause of oral cavity pain and tooth loss^[2]. Toothpaste can be used as an ingredient to prevent dental caries in the oral cavity^[3-5]. Toothpaste is a paste or gel used to maintain and improve oral health and esthetics^[6]. Its composition consists of a combination of various compounds that function to eliminate dental caries^[7-9]. When this has been widely developed, natural ingredients are used as medicine, one of which is *Allium sativum*.^[1,10]

Research by Belguith et al.^[11] found that garlic shows antibacterial activity against various gram-positive and gram-negative bacteria, including species *Escherichia*, *Salmonella*, *Staphylococcus*, *Klebsiella*, *Proteus*, *Bacillus*, *Clostridium*, *Neisseria*, *Proteus*, *Pseudomonas*^[12]. The main compound contained in *Allium sativum* as an antibacterial activity is allicin. The allicin compound is produced after the intact *Allium sativum* tissue is destroyed or extracted. Then alliin is converted to allicin by the enzyme alliinase^[13]. The results of previous studies indicate that the antibacterial activity of garlic is mainly

due to the compound allicin. Antibacterial activity of allicin against *Streptococcus mutans*^[14], *Streptococcus sanguis*, *Streptococcus salivarius*, *Pseudomonas aeruginosa* and *Lactobacillus* spp. at concentrations of 5%, 10%, 20%, and 100%^[15].

Lactobacillus acidophilus bacteria are pathogenic bacteria that cause dental caries and productive lactic acid producers and are acid-tolerant^[16]. These bacteria are often found isolated from active caries. *Lactobacillus acidophilus* is considered a secondary invading bacterium, not a bacterium in the process of invading dental caries because *Lactobacillus acidophilus* appears after caries lesions are formed. *Lactobacillus acidophilus* predominates in advanced carious lesions in adults, even more than *Streptococcus mutans*. *Lactobacillus acidophilus* in severe early childhood caries (ECC) in children is found not as a dominant genus such as adult caries lesions. Habitats in the oral cavity are found in hollows such as in dental caries and dental fissures^[17]. *Lactobacillus acidophilus* bacteria cannot be attached directly to tooth enamel, but in collaboration with *Streptococcus mutans*, a trigger in the production of lactic acid which is responsible for the demineralization process of tooth enamel^[18].

Based on the background that has been described, the researchers are interested in

conducting research on the inhibition of allicin in toothpaste on Lactobacillus Acidophilus.

MATERIAL AND METHOD

The research conducted was an experimental laboratory study with a post-test only control group design. In this study, allicin added to toothpaste was divided into 4 groups, namely the control group, and the treatment group consisting of aquades, toothpaste without allicin, toothpaste containing 5% allicin, toothpaste containing 10% allicin, paste teeth containing allicin 20%, and toothpaste containing allicin 40%. Repeat the sample 4 times. The factor measured or seen was the inhibition of allicin toothpaste against Lactobacillus Acidophilus.

Toothpaste containing allicin is made in several stages. To get allicin, freshly harvested garlic, then weighed using a 500 gram digital scale. Garlic that has been weighed and then washed using running water until clean and then drained. Garlic is cut using a chopper then mashed with a blender. Garlic that has been mashed weighed as much as 500 grams, then macerated by leveling the garlic that has been mashed in a jar with 70% alcohol solvent, to cover the entire surface of the base of the jar and submerged garlic. The total 70% alcohol used is as much as 1000ml.

The jar is tightly closed, and left for 24 hours, then shaken using a digital shaker with a speed of 50 rpm. Garlic that has been soaked in 70% alcohol solvent is then filtered using a cloth filter and placed in an Erlenmeyer tube. Garlic is macerated 2 times by putting garlic pulp back into the jar, then adding 70% alcohol solvent until submerged (at least 5cm solvent above the surface of the garlic). Furthermore, it is left for 24 hours and shaken using a digital shaker.

Each remaseration used a 70% alcohol as much as 1000ml. Remaseration is done until the garlic extract filtrate results are clearer (repeated 2 times). The results of the first garlic extract until the final garlic extract results are then combined into one and evaporated using a rotary evaporator and waterbath at a temperature of 60 ° C until all alcohol solvents separate (approximately takes 3 hours 30 minutes) so that no residual alcohol is removed still contained in the garlic extract. From the garlic which was weighed using a 500 gram digital scale then extracted using 70 ml of 70% alcohol solvent, resulting in an extract of garlic (allicin) of 70 ml.

The second step is to make toothpaste containing allicin by putting calcium carbonate (CaCO₃) into the mortar, then stirring it until smooth and homogeneous using pestle. After being homogeneous, Sodium Carboxymethyl Cellulose (CMC Na) and SLS are put into a

mortar. Then crushed and ground again using a pestle. Put 20% water until dough becomes paste. Stirring was conducted by smooth and homogeneous for 5 minutes at room temperature (27 °C). The toothpaste dough is then placed in a closed package. Allicin was stirred with the paste until homogeneous for 5 minutes at room temperature (27 °C). Toothpaste dough containing allicin is placed in a closed place.

The third stage is the preparation of Lactobacillus Acidophilus bacteria. Lactobacillus Acidophilus culture that will be used is taken from Lactobacillus Acidophilus stock using sterile osse and planted on BHIB media. Lactobacillus Acidophilus is cultured in an anaerobic jar with anaerobic atmosphere and incubated for 24 hours at 37°C. The culture was matched with the Mc Farland standard, then thinned to the Mc Farland standard of 0.5 or equivalent to 1.5 x 10⁸ CFU / ml to obtain bacteria with a certain density. Equating the turbidity of a bacterial suspension by double blind is done by holding the test tube side by side and observing on a white background with black stripes. If the turbidity of the bacterial suspension is still not the same, the bacterial suspension can be diluted or given additional bacteria. After obtaining the same turbidity as the standard, the suspension is diluted. To inoculate the Lactobacillus Acidophilus bacteria in the Agar Nutrient Media, prepare 2 petri dishes containing nutrient agar which are divided into several sections (2 control groups and 4 treatment groups). Taking the bacteria Lactobacillus Acidophilus from BHIB, which has been equaled with turbidity 0.5 Mc Farland solution. Rub the culture onto the entire surface of the nutrient agar using a sterile cotton swab.

The fourth step was to test the inhibition of toothpaste containing allicin against bacteria. The inhibitory test in this study used the Agar Plate Diffusion method. Lactobacillus Acidophilus is taken from a test tube using a swab, spreading it on agar nutrient media. Making wells in nutrient agar media that has been prepared. Basic toothpaste and toothpaste containing allicin are included in the well. Petri dishes are incubated at 37 ° C for 48 hours. How to measure by turning petridish with the base facing up and the petridish lid not being lifted so that the inhibition zone looks clear in the area around the well that indicates the presence of bacterial growth inhibition. Inhibition zone diameters are calculated using calipers with a level of accuracy of 0.1 mm and results are expressed in millimeters (mm).

RESULT

The observations were made in the control group and the treatment group consisting of aquades, toothpaste without allicin, toothpaste containing 5% allicin, toothpaste containing 10% allicin,

toothpaste containing 20% allicin and toothpaste containing 40% allicin . The study was conducted by observing the inhibition of allicin in toothpaste on Lactobacillus Acidophilus.

Table 1: Sample size, mean value and standard deviation of allicin toothpaste against Lactobacillus Acidophilus.

Care Group	Number of Samples	Average	Standard deviation
K1	4	0	0,500
K2	4	0	0,957
P1	4	0	1,414
P2	4	0	1,892
P3	4	10,72	0,783
P4	4	23,69	2,027

From the graph it can be seen that distilled water, toothpaste without allicin, toothpaste containing 5% allicin, toothpaste containing 10% allicin have no inhibitory power against Lactobacillus Acidophilus, while the toothpaste containing allicin 20% have minimal inhibition against Lactobacillus Acidophilus and Toothpaste containing 40% allicin has the most effective inhibition against Lactobacillus Acidophilus.

To find out the difference in the influence of distilled water, toothpaste without allicin and toothpaste containing allicin 5% concentration; 10%; 20%; 40%; on the inhibitory value of Lactobacillus Acidophilus data analysis was performed. Previously conducted a normality test

using the Kolmogorov-Smirnov Test which states that the data is distributed in each group normally if the data shows a significance value or $p > 0.05$. These results indicate that $p > 0.05$ so that said data are normally distributed.

In the results of the study, it was seen that the average of distilled water, toothpaste without allicin, toothpaste containing 5% allicin, toothpaste containing allicin 10% had no change when the average toothpaste containing allicin 40% was higher than toothpaste containing 20% allicin . The standard deviation value is seen that is increasing. Graph of inhibition zone values and with allicin concentration.

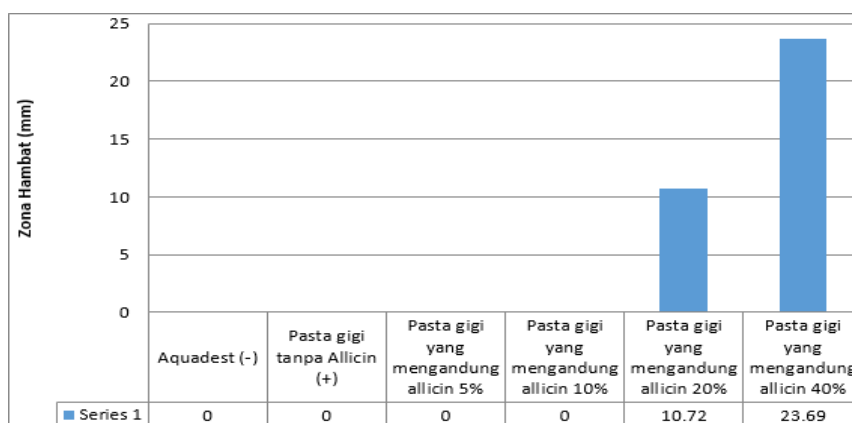


Fig.2: Graph of inhibition zone relationship and allicin concentration

Data analysis continued with Levene's test. This test aims to evaluate homogeneity or to discover whether or not several groups of research data have the same variant or not. In other words, homogeneity means that the data set under study has the same characteristics. The Levene's Test states that the data can be homogeneous if the significance value or $p > 0.05$. These results indicate that $p > 0.05$ included in homogeneous data.

Requirements for conducting One-way Anova Test are research data should be normally distributed and homogeneous. Based on the results of the Kolmogorov-Smirnov Test and Levene's test, shows that the datas are normally distributed and homogeneous, are eligible for the One-way Anova Test. This test was conducted to determine the existence of significant differences in all treatment groups and control groups. The research data shows a significant

difference if the probability value is less than 0.05 ($p < 0.05$).

The results of the One-way Anova Test, showed a significant difference in the sample group, so it was necessary to do a Tukey HSD test to determine significant differences between the sample groups. The results of the Tukey HSD test data show a significant difference if the probability value is equal to 0.05 ($p < 0.05$).

DISCUSSION

Lactobacillus acidophilus bacteria are pathogenic bacteria that cause dental caries and produce lactic acid which is productive and acid tolerant. These bacteria are often found isolated from active caries^[16]. Prevention of bacteria that cause dental caries is by brushing teeth using toothpaste^[3].

Research has been conducted to show the inhibitory power of allicin in toothpaste against Lactobacillus Acidophilus bacteria. The study was conducted by using distilled water as a negative control, toothpaste without allicin as a positive control and allicin with a concentration of 5%, 10%, 20% and 40% mixed in the bottom of the toothpaste as a treatment. The results showed that distilled water, toothpaste without allicin, toothpaste containing 5% allicin and toothpaste containing 10% allicin did not have any inhibitory effect on each lactobacillus acidophilus test. Toothpaste, which contains 20% allicin and has minimal inhibition, toothpaste containing 40% allicin has the greatest inhibition in the research that has been done. The test results showed that the inhibition zones of concentration in distilled water, toothpaste without allicin, toothpaste containing 5% and 10% allicin were not significantly different, but the inhibitory power of toothpaste containing allicin concentrations of 20% and 40% were significantly different. This condition shows that the higher the concentration, the higher the inhibition of bacteria.

The cause of the inhibition of Lactobacillus Acidophilus bacteria is because the main compound contained in allium sativum as an antibacterial activity is allicin^[12]. The allicin compound is produced after fresh allium sativum tissue is destroyed or extracted. In the extraction process alliin is converted to allicin by the enzyme allinase^[13]. The way the antibacterial activity works is by inhibiting the thiol enzyme in allium sativum which runs the reaction quickly so that allicin can penetrate the phospholipid bilayers of bacterial cell walls^[19], then inhibits the synthesis of nucleic acids and proteins, deoxyribonucleic acid (DNA), Ribonucleic acid (RNA). Damage to the cell wall by allicin can cause the metabolic system to be disrupted^[20] so that bacterial cell

growth is inhibited and even results in death of bacterial cells because organelles present in bacteria cannot function. The minimum inhibitory level and the minimum kill rate of allium sativum will be higher if the allicin concentration is high^[21].

This is consistent with research conducted by several researchers showing that the antibacterial activity of garlic is mainly due to the compound allicin. The results showed the antibacterial activity of allicin at concentrations of 5%, 10%, 20%, and 100% against Streptococcus mutans, Streptococcus sanguis, Streptococcus salivarius, Pseudomonas aeruginosa, and Lactobacillus spp. The test results showed inhibition zone concentrations at 5%, 10% and 20% did not differ significantly, but the inhibition was better at a concentration of 100%^[15].

Increasing the concentration of allicin extract will increase inhibition against lactobacillus acidophilus, this is in accordance with Irnawati's statement that the concentration of antibacterial substances is a factor that inhibits microorganisms by antibacterial agents. The higher the concentration of anti-bacterial substances, the more bacteria are blocked or dead^[22].

CONCLUSION.

Lactobacillus Acidophilus is better inhibited by allicin in toothpaste with a higher concentration.

CONFLICT OF INTEREST

None

SOURCE OF FUNDING

Self-Funding

ETHICAL CLEARANCE

This study was approved by the Universitas Airlangga, Faculty of Dental Medicine Health Research Ethical Clearance Commission

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