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POTENTIONAL OF SINGLE GARLIC EXTRACT ON *STREPTOCOCCUS MUTANS* ATCC® 21752™ (IN VITRO)

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ABSTRACT

Effort for controlling *Streptococcus mutans* is using antibacterial ingredients, type of laboratory experimental research design with *post-test only control group design*. Testing bacteria in this study used the diffusion method with disc, despite garlic extract (*Allium sativum*) was first diluted with DMSO into four concentrations, that was 20%, 40%, 60%, and 80%. The positive control was amoxicillin and the negative control was DMSO. Data analysis used one way ANOVA statistical test which aims to see differences in the antibacterial effect of four concentration and control group; amoxicillin and DMSO. The data analysis was continued with the *Post Hoc* (LSD) test to see the difference in the antibacterial effect between the four concentration and control group. The results showed that the inhibition zone of single garlic extract of four concentration against *Streptococcus mutans* ATCC® 21752™ was 6.95 ± 0.13 mm; 7.57 ± 0.17 mm; 8.60 ± 0.14 mm; and 11.12 ± 0.17 mm. The conclusion of this study, there are significant differences in several concentrations, the 80% strongest zone of inhibition zone was 11.12 mm and the 20% medium category was 6.95 mm. The concentration of 80% is the most effective concentration in inhibiting the growth of *Streptococcus mutans* bacteria.

Keywords: *Streptococcus mutans* ATCC® 21752™, antibacterial, single garlic, extra

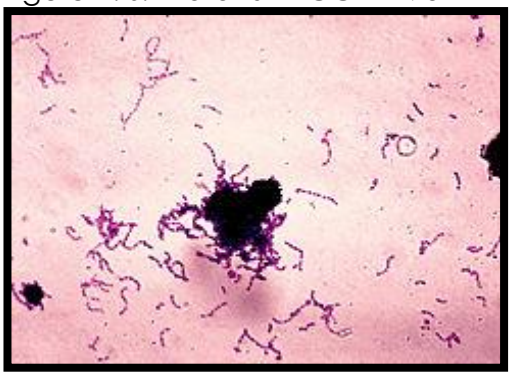
INTRODUCTION

Caries prevalency are high enough in Indonesia, this means that is the main problem in dentistry. Caries is still a problem in dentistry and in Indonesia with a fairly high prevalence.[1] Based on the Basic Health Research (RISKESDAS) in 2018, the overall value of the Indonesian DMFT Index is 7,1, which means tooth decay of the Indonesian population is 710 teeth per 100 people.[2]

Caries or cavities is a disease caused by damage to the enamel layer that can extend to the nerves of the teeth caused by bacterial activity in the mouth. Caries is a disease of the hard tissue of teeth, namely enamel, dentin and cementum, which undergoes a regressive chronic process.[3] Bacteria that play a role in caries formation include *Streptococcus mutans* and are gram-positive, nonmotile and facultative anaerobic that can metabolize carbohydrates, under the microscope with gram staining purple and arranged like a chain.[4]

Streptococcus mutans was first isolated by researcher J. Killian Clarke in 1924, is a normal flora which is known as the main bacteria causing dental caries. These bacteria can turn into pathogens when their population increases, so control of their growth is very important to prevent caries.[5]

Figure 1. *S. mutans* ATCC® 21752™



(Documentation)

Streptococcus mutans is facultative anaerobic because it grows both in an atmosphere with oxygen and without oxygen. In anaerobic conditions these bacteria require 5% CO2 and 95% nitrogen and require ammonia as a nitrogen source in order to survive in a thick layer of plaque.[6]

Streptococcus mutans is acidogenic, that is, it has the potential to produce acid and is acidic, it can live in an acidic environment. In the growth hour period in the broth, the terminal pH of *Streptococcus mutans* was 3,4. *Streptococcus mutans* is able to produce extracellular polysaccharides which facilitate its attachment to the tooth surface so that it can adhere to the components on the tooth surface. This interaction causes a decrease in pH and accelerates the demineralization and allows the occurrence of caries.[6,7]

Streptococcus mutans produces two enzymes is glycosyltranfirase and fructosyltransferase. These enzymes are specific to the substrate, sucrose, which is used to synthesize high molecular weight glucans and fructants. Glucans bind to special receptors on the surface of *Streptococcus mutans*, this reaction occurs when these bacteria are cultured on media containing sucrose. *Streptococcus mutans* bacteria utilize sucrose from food to increase bacterial colonization. The production of glucans from sucrose by enzymes produced by *Streptococcus mutans* is an important step in the production of caries.[6]

Efforts to control *Streptococcus mutans* use antibacterial ingredients. Antibacterials are substances that can inhibit or kill pathogenic bacteria that cause infection. Single garlic (*Allium sativum*) is a medicinal plant that has the potential to be antibacterial.[8]

This effect is due to the fact that single garlic contains active compounds, namely allisin, flavonoids, saponins and tannins which can denature proteins, damage cell membranes, damage permeability of cell walls, microsomes, lysosomes and do not occur colonization.[8,9]

All of the active ingredients contained in garlic have antibacterial properties. The content of active compounds contained in single garlic is alicins, flavonoids, saponins and tannins. These active compounds work synergistically as antibacterials by damaging cell walls and lysing bacterial cells, and inhibiting proteolytics.[10]

Alisin and its derivatives namely *Diallyl disulfide* (DADS) and *Diallyl sulfide* (DAS) can inhibit gram positive and gram negative bacteria by inhibiting RNA production and lipid synthesis. This inhibition causes amino acids and proteins cannot be produced and the phospholipid bilayer of the cell wall cannot be formed, so that growth and development in bacteria will not occur.[10]

Alisin compounds increase the permeability of the bacterial wall which causes the SH groups (*sulfihydrils* and *disulfides*) to be destroyed in the amino acids cystine and cysteine. The destroyed SH group inhibits the synthesis of the protease enzyme, which damages the cytoplasmic membrane of the bacterial wall and disrupts the metabolism of proteins and nucleic acids, causing proliferation in bacteria. The flavonoids contained in single garlic cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes as a result of the interaction between flavonoids and bacterial DNA.[9,10,11]

Tannins are phenol-derived compounds found in single garlic. Tannins can bind to bacterial cell walls, inhibit metabolic processes and protein-forming activities and are toxic to bacteria. Tannins inhibit bacterial growth by denaturing proteins and reducing surface tension, so that bacterial permeability increases. Damage and increased permeability of bacteria causes stunted cell growth and leads to cell death. Tannins can inhibit proteolytics which play a role in breaking down proteins into amino acids so that it will interfere with bacterial cells in absorption of protein by cell fluids and do not occur colonization.[9,11]

Saponins can interfere with the process of forming bacterial cell membranes by creating pemeability so that cell division does not occur. Saponins are antibacterial, which can cause protein and enzyme leakage from within cells. The mechanism of action of saponins as antibacterials is to reduce surface tension resulting in increased permeability or cell leakage and resulting in intracellular compounds to be released. Saponins diffuse through the outer membrane and susceptible cell walls and then bind to the cytoplasmic membrane thereby disrupting and reducing cell membrane stability. This causes the cytoplasm to leak out of the cell resulting in cell death.[9,11]

Figure 2. Single garlic (*Allium sativum*) (Documentation)

In Reni's study, the average garlic extract had an inhibition zone of 11-15 mm against the *Porphyromonas gingivalis* bacteria.[8] Pratimi's research found that the bacteriostatic potential of single garlic compared to compound garlic was higher due to the ratio of the active compound content in one piece of garlic. single is equivalent to 5-6 cloves of garlic.[13,14]

Based on the description above, single garlic (*Allium sativum*) has the potential as an antibacterial medicinal plant. This study aims to test the antibacterial activity with various concentrations of single garlic extract against the bacterium *Streptococcus mutans* ATCC® 21752™ in vitro.

RESEARCH METHODS

This type of research is a laboratory experimental with post test only control group design. The sample of this study was an isolate of *Streptococcus mutans* ATCC® 21752™. The sample size was determined by Federer's formula and obtained 4 samples for each group, so that the total sample for 6 groups was 24.

The work procedure in this study includes the following stages:

1. Making single garlic extract.
2. Making *Mueller Hinton Agar* (MHA).
3. Testing of single garlic extract on *Streptococcus mutans* ATCC® 21752™.

The data obtained were analyzed using descriptive test, oneway ANOVA and posthoc LSD.

RESULT

Determination of inhibitory zone by measuring the diameter of clear zone around the paper discs using a digital caliper with an accuracy of 0.01 mm. Based on the results of garlic extract single concentration of 20%, 40%, 60%, and 80% found their inhibition zone diameter on *Streptococcus mutans* ATCC® 21752™ bacteria (Table 1).

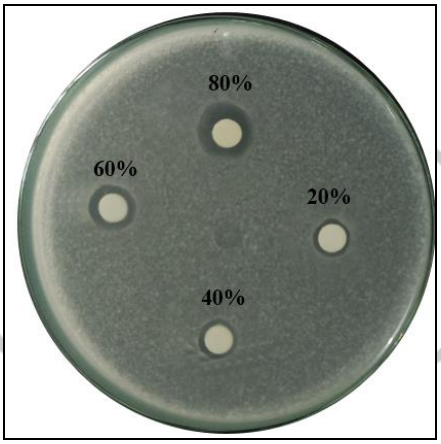


Figure 3. Inhibition zone of single garlic extract on *S.mutans* ATCC® 21752™ (Documentation)

Table 1. Inhibition zone diameter of single garlic extract on *Streptococcus mutans* ATCC® 21752™

Consentration	I	II	III	IV	Mean ±SD
20%	6,9	7,1	6,8	7,0	6,95±0,13
40%	7,5	7,8	7,4	7,6	7,57±0,17
60%	8,6	8,5	8,8	8,5	8,60±0,14
80%	11,1	10,9	11,3	11,2	11,12±0,17
K^+	17,6	17,4	17,7	17,6	17,57±0,13
K^-	6,0	6,0	6,0	6,0	6,00±0,00

The results of the study in table 1 obtained a single garlic extract with a concentration of 20%, 40%, 60%, and 80% and a positive control found an inhibition zone in the *Streptococcus mutans* ATCC® 21752™ bacteria with different amounts in each replication.

The mean value of single garlic extract inhibitory diameter in inhibiting the growth of the bacteria *Streptococcus mutans* ATCC® 21752™ concentrations of 20%, 40%, 60%, and 80% and the positive control was 6.95 ± 0.13 mm; 7.57 ± 0.17 mm; 8.60 ± 0.14 mm; 11.12 ± 0.17 mm; and 17.57 ± 0.13 mm.

One way ANOVA test was used to see the antibacterial effect of a single garlic extract on the growth of the bacteria *Streptococcus mutans* ATCC® 21752™ (Table 2)

Table 2. One way ANOVA test results of single garlic extract in inhibiting the growth of the bacteria *Streptococcus mutans* ATCC® 21752™

Group	*Streptococcus mutans*		
	Mean	SD	*p value*
20%	6,95	0,13	0,000
40%	7,57	0,17	
60%	8,60	0,14	
80%	11,12	0,17	
K^+	17,57	0,13	
K^-	6,00	0,0	

Based on the results of the one way ANOVA test, the significance value of p = 0,000 means that there is a difference in inhibition diameter of all treatment groups in the *Streptococcus mutans* bacteria at a = 5%. This value indicates that a single garlic extract with a concentration of 20%, 40%, 60%, 80% is able to inhibit the growth of the bacteria *Streptococcus mutans* ATCC® 21752™.

After the one way ANOVA statistic test, then followed by the *posthoc* LSD test which aims to see the difference in antibacterial effects between the two treatment groups on the bacteria *Streptococcus mutans* ATCC® 21752™. The results of the posthoc test with LSD in all treatment groups showed that there was

a significant difference in inhibiting the growth of the bacteria *Streptococcus mutans* ATCC® 21752™.

DISCUSSION

1. Antibacterial Effect of Single Garlic Extract Concentration of 20%, 40%, 60%, 80% against Streptococcus mutans ATCC® 21752TM

Based on the results of the oneway Anova test, the p value was obtained= 0.000, which means that there is a difference in the average inhibition zone diameter of a single garlic extract at a concentration of 20%, 40%, 60% and 80% and a positive control against *Streptococcus mutans* ATCC® 21752™. Supported by the results of the posthoc LSD test.

From the research results it can be stated that there is an antibacterial effect of single garlic extract (*Allium sativum*) at a concentration of 20%, 40%, 60% and 80% against *Streptococcus mutans* ATCC® 21752™. In line with the research of Sutiyono et al that garlic extract effectively inhibits the growth of the *Aggregatibacter actinomycetemcomitans* bacteria that causes gingivitis with a 50% concentration of 11.50 mm.[15]

These results are also in line with the research conducted by Borhan-Mojabi *et al*, where a concentration of 40% was able to inhibit the growth of *Stresptococcus mutans* bacteria. However, the method of making antibacterial testing in the study of Borhan-Mojabi *et al* is different from this study. Testing the antibacterial effect in the study of Borhan-Mojabi *et al* used the dilution method, while this study used the diffusion method.[16]

The formation of an inhibition zone in *Streptococcus mutans* ATCC® 21752™ is due to the presence of certain substances in the single garlic extract that are antibacterial. The ability of single garlic extract (*Allium sativum*) as an antibacterial comes from the chemical compounds contained therein. Single garlic extract contains active antibacterial compounds such as allisins, flavonoids, saponins and tannins.[17,18]

Allisin, a sulfur derivative, can damage cell walls and inhibit protein synthesis. Saponins and flavonoids contained in garlic can also damage bacterial cell membranes. In addition, tannins can inhibit proteolytics, which play a role in breaking down protein into amino acids so that it will interfere with bacterial cells in the absorption of protein by cell fluids and colonization does not occur.[20] These active compounds work synergistically as antibacterials by damaging cell walls and lysing bacterial cells, and inhibit proteolytic.[9]

2. Inhibitory Diameter of Single Garlic Extract Concentration of 20%, 40%, 60%, 80% against Streptococcus mutans ATCC® 21752™

Traditional medicine has been widely used by society today. One of the natural plants that have begun to be developed in dentistry as an antibacterial agent is garlic.[19,20] Testing the antibacterial effect of a single garlic extract aims to obtain the diameter of the inhibition zone. The diameter of the inhibition zone can be seen from the clear zone formed around the disc which has been contaminated by the bacteria *Streptococcus mutans* ATCC® 21752™. The calculation of the inhibition zone diameter after incubation for 24 hours.

The effectiveness of the antibacterial activity can be seen from the inhibition zone formed.[21] Based on the results of the study, it was found that the average diameter of the inhibition zone of a single garlic extract at a concentration of 20%, 40%, 60% and 80% was 6.95 ± 0.13 mm ; 7.57 ± 0.17 mm; 8.60 ± 0.14 mm; and 11.12 ± 0.17 mm. The mean diameter of the inhibition zone for positive control was 17.57 ± 0.13 mm, and for negative control was 6.00 ± 0.00 mm. 80% concentration is the most effective concentration in inhibiting the growth of *Streptococcus mutans* bacteria because it is close to the inhibition zone diameter + control.

Andayani *et al* research stated that a single garlic extract can inhibit the growth of *Candida albicans* at a concentration of 100%, 80%, 60%, 40% with an average diameter of the inhibition zone of each concentration is 21.4 mm, 18.6 mm, 14,8 mm, and 11.6 mm. However, a concentration of 20% did not produce an inhibition zone. From these results it

can be seen that the single garlic extract not only has an inhibition zone in bacteria, but this extract also has inhibition in fungi.[22]

Davis and Stout explained the classification of bacterial growth inhibition responses based on the clear zone diameter consisting of 4 groups, namely low (diameter ≤5 mm), moderate (5-10 mm diameter), strong (10-20 mm diameter), and very strong responses. (diameter ≥20 mm).[21] Based on the classification, the results of the single garlic extract group with a concentration of 80% provided a strong inhibitory power, while other concentrations of single garlic extract, namely concentrations of 60%, 40% and 20%, provided moderate inhibition.

The antibacterial activity of garlic has a broad spectrum, effective against gram positive (+) and gram negative (-) bacteria.[22] The effectiveness of antibacterials is influenced by the concentration of these substances. The increase in the concentration value of garlic extract is directly proportional to the size of the formed inhibition zone. This means that the increasing the concentration of the extract, the greater the inhibition zone formed.[23]

The greater the concentration of a single garlic extract, the stronger the inhibitory power against *Streptococcus mutans* ATCC® 21752™. Thus, the lower the concentration of a single garlic extract, the weaker the inhibition against *Streptococcus mutans* ATCC® 21752™. This is because the active substances contained in a single garlic become less so that the resulting zone of inhibition is smaller. In addition, the diameter of the formed inhibition zone is influenced by factors such as the turbidity of the bacterial suspension, the time of impregnation, the bacterial suspension into the MHA media, the incubation temperature, the incubation time, the thickness of the media and the composition of the media.

CONCLUSION

Based on the results of the study it can be concluded that the inhibition zone of single garlic extract (*Allium sativum*) at concentrations (20%, 40%, 60%, and 80%) against *Streptococcus mutans* ATCC® 21752™ is 6.95 ± 0.13 mm; 7.57 ± 0.17 mm; 8.60 ± 0.14 mm; and 11.12 ± 0.17 mm. The concentration of 80% is the most effective concentration in inhibiting the growth of *Streptococcus mutans* bacteria.

SUGGESTION

1. It is hoped that the next researchers will use a single garlic extract (*Allium sativum*) with various concentrations and other test methods to inhibit the growth of *Streptococcus mutans*.
2. It is recommended that further researchers use a single garlic extract (*Allium sativum*) at concentrations (20%, 40%, 60%, and 80%) to inhibit the growth of other oral bacteria.

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