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INHIBITION OF GARLIC EXTRACT (*ALLIUM SATIVUM*) IN 50% CONCENTRATION TO *STAPHYLOCOCCUS AUREUS* BACTERIA (*IN VITRO*)

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ABSTRACT

Objective: Periodontal abscess is an inflammatory process that occurs due to a localized bacterial infection involving deeper periodontal structures and may occur acutely or chronically. One of the bacteria that causes periodontal abscess is *Staphylococcus aureus*. Periodontal abscesses is generally treated using Amoxicillin, but it can appear resistant requiring an alternative by utilizing natural ingredients such as garlic (*Allium sativum*). Garlic has an allicin active substance that has efficacy as an antibacterial. The objective of this study was to determine the inhibition of garlic extract with a concentration of 50% against *Staphylococcus aureus* bacteria *in vitro*.

Methods: The research method is using experimental laboratory research with post-test design group *in vitro* where the inhibitory test was used the agar disc diffusion method (Kirby Bauer) with garlic extract (*Allium sativum*) with the concentration of 50%, and Amoxicillin 30 µg positive control (CT0223B). Culture media is using Mueller Hinton Agar (MHA).

Results: The results of the experiment on the inhibition of garlic extract (*Allium sativum*) showed that the average of the inhibitory zone after treatment in both groups was significantly different ($p<0.05$). There was a difference of inhibition zone between the control group (Amoxicillin 30 µg (CT0223B)) and the treatment group (garlic extract with concentration of 50%) where the average of the control group's inhibitory zone (Amoxicillin 30 µg (CT0223B)) was greater than the treatment group (garlic extract (*Allium sativum*) concentration of 50%).

Conclusion: There is inhibition of garlic extract (*Allium sativum*) with a concentration of 50% against *Staphylococcus aureus* bacteria *in vitro*.

Keywords: Barrier power, Garlic extract, *Allium sativum*, *Staphylococcus aureus*

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INTRODUCTION

The prevalence of the periodontal disease is still quite high, not only in Indonesia but in many developing countries. Periodontal disorders ranks second after dental caries as a cause of tooth loss in adults in developing countries. Periodontal disease can be defined as a pathological process involving periodontal tissue. Periodontal inflammation is largely due to bacterial infections, the primary cause of the periodontal disease is the microorganisms that colonize on the tooth surfaces, although there are still other causal factors [1]. Periodontal disorders suffered in general are periodontitis and abscess. The most common abscess of the oral cavity is a periodontal abscess and periapical abscess [2]. The periodontal abscess is an inflammatory process due to a localized bacterial infection involving deeper periodontal structures and may occur acutely or chronically [1]. Approximately 60% of bacteria encountered are anaerobic bacteria known to be pathogenic against periodontal, such as *Stapylococcus aureus, Porphyromonas gingivalis, Provotella intermedia* and *Fusobacterium nucleatum* [2, 3]. One of the pathogenic bacteria that causes periodontal abscesses that often infect humans is *Staphylococcus aureus. Staphylococcus aureus* bacteria are positive coagulase, which distinguishes them from other types of bacteria [4, 5].

Common antibiotics that used to treat infections caused by gram-positive bacteria are Amoxicillin, which is a broad-spectrum antibiotic. Amoxicillin is also highly effective against most periodontal pathogens and shows high levels of antimicrobial activity achieved in gingival sulcus [6]. *Staphylococcus* genus bacteria are rapidly becoming resistant to many antimicrobial drugs and cause difficult therapeutic problems [4]. *Staphylococcus aureus* is one of the bacteria with high resistance to various antibiotics in Indonesia. *Staphylococcus aureus* has been shown to be resistant to penicillin, oxacillin, and other beta-lactam antibiotics [7]. To solve the problem, one of the long-established efforts in the last decades is to take alternative pathways using natural plant-based medicines [8].

The use of garlic (*Allium sativum*) therapeutic has long been known to have the potential for the treatment of infections of various organisms. Previous research has focused only on the antibacterial effects of garlic (*Allium sativum*) on *Staphylococcus aureus*. Much of the literature suggests that Gram-positive bacteria *Staphylococcus aureus* is said to be more susceptible to the toxic effects of garlic (*Allium sativum*) than gram-negative bacteria [9].

The choice of garlic (*Allium sativum*) due to its daily use is often used as an alternative medicine to cure ulcers on the skin, which is often caused by gram-positive bacteria, *Staphylococcus aureus* [9]. Garlic (*Allium sativum*) is also very easy to find among the daily life and is a plant that is almost always found in the every kitchen of each home. Based on the problem, the researcher wanted to know the inhibition of garlic extract (*Allium sativum*) concentration 50% against *Staphylococcus aureus* bacteria *in vitro*.

Garlic (*Allium sativum*) generally grows in the highlands, but certain varieties are able to grow in the lowlands. Sandy clay or dusty clay-textured soil with neutral pH becomes a good growing medium. The land of this plant should not be inundated waterlogged. Suitable temperatures for upland cultivation range from 20-25 °C with rainfall of about 1.200-2.400 mm per year, while temperatures for lowlands range from 27-30 °C [10].

The content of chemical compound from garlic bulb (*Allium sativum*) per 100 gram is, Alisin (1.5 g) which is important component with antibiotic effect, protein (4.5 g), fat (0.20 g), hydrate of charcoal (23.1 g), vitamin B1 (0.22 mg), vitamin C (15 mg), calories (95 g), posfor (134 mg), calcium (42 mg), iron (1 mg), and water (71 g) [11].

Staphylococcus aureus is a round-shaped Gram-positive bacterium 0.7-1.2 µm in diameter, arranged in irregular groups like grapes, does not require atmospheric oxygen but grows better in its environment (facultative anaerobes), does not form spores, and not moving, this bacterium grows at an optimum temperature of 37 °C, but forms the best pigment at room temperature (20-25 °C). The colour of colonies on solid seed grey to golden yellow, round, smooth, protruding, and shiny [12].

Periodontal abscess is an inflammation that occurs due to a localized bacterial infection of the periodontium tissue. This lesion is also called a lateral periodontal abscess or a parietal abscess. Periodontal

abscesses are known to be lesions that can rapidly damage the periodontium tissue occurring over a limited period of time as well as easily known clinical symptoms and signs such as local accumulation of pus and located within the periodontal pocket [13].

Clinical features of a periodontal abscess, seen as slippery, gingival swelling with pain, gingival swelling areas are soft because of purulent exudates and increased probing depth, teeth become sensitive when exposed and may become rapidly mobilizing and rapidly attaching periodontal attachment [13].

Periodontal abscess is an emergency case at the dental clinic, where the most frequent third periodontal disease reaches 7-14%, after acute dentoalveolar abscess (14-25%) and pericoronitis (10-11%) [12]. The prevalence of cases of periodontal abscesses is relatively high and affects the prognosis of the teeth, especially in patients with periodontitis. Patients with periodontal abscesses are more likely to occur in pre-existing periodontal pockets [13].

MATERIALS AND METHODS

Material

The research used is experimental laboratory research with the post-test design group. The samples used in the study were colonies of *Staphylococcus aureus* bacteria (ATCC 25922) while the treatments were divided into 2 groups based on preliminary studies where concentrations of 50% garlic extract and Amoxicillin 30µg (CT0223B).

The tool used in this research is scales, blenders, Erlenmeyer, Buchner funnel, rotary evaporator, petri dish, paper disk blank, blue tip, yellow tip, micropipette, sterile cotton swab, Bunsen lamp, incubator, filter paper, aquades, gloves, aluminum foil, label paper, thrust, timer, glass tube, ose, tweezers, water bath, while materials used, garlic (*Allium sativum*), ethanol 96%, blood agar vm458486 (Merck), Mueller-Hinton agar vm371937, NaCl 0.9%, *Staphylococcus aureus* ATCC 25922, amoxicillin 30µg (CT0223B). Garlic (*Allium sativum*) was peeled and weighed (500 g), and then sliced into three parts. The garlic slices were fed into the oven at 40 °C and then removed from the oven and fed into the crusher (powder maker).

Garlic extract was prepared by weighed 80 g of garlic powder and dissolving in 600 ml of 96% ethanol. The mixture was put into a dark bottle, stirred, and then closed and kept for 3 d. The result of the immersion is filtered using a cloth and the liquid extract was concentrated by insertion into the oven at 40 °C until the liquid is obtained. The concentrated extract was placed in a sterile container and made into a 50% concentration test solution. A 50% solution means the solution comprises 50 ml of garlic extract and 50 ml of distilled water. Preparation of the negative control solution is by using disc containing antibiotic amoxicillin 30 µg (CT0223B).

The 99.5 ml H_2SO_4 0.36N solution was mixed with a 1.175% $BaCl_2.2H_2O$ solution with a turbidity of 0.5Mc. Farland equivalent to 108 CFU/ml in an Erlenmeyer. The mixture was shaken until a cloudy solution is formed. This turbidity is used as standard turbidity of bacterial suspension test. The suspension of *Staphylococcus aureus* ATCC 25922 equivalent to 108 CFU/ml, taken with a sterile cotton swab. Then applied evenly over the medium of Mueller Hinton Agar sterile. Garlic extract (*Allium sativum*) with concentration 50%, and positive control is added blank disk of 6 seeds. Then the disk containing garlic extract with a concentration of 50%, and positive control was placed on the Mueller Hinton agar medium containing the suspension of *staphylococcus aureus* ATCC 25922, and incubated in the incubator at 37 °C for 24 h.

Observations were made after 24 h of incubation period. Clear areas are an indication of bacterial susceptibility to antibiotics or other antibacterial agents that can be used as test materials expressed to the width of the inhibitory zone diameter. The drag zone diameter is calculated in millimeters (mm) using the slider. Then the diameter of the inhibit zone is categorized as antibacterial power strength based on the classification of Davis and Stout, i.e. as follows, the clear zone diameter ≥ 20 mm means very strong inhibitory, clear zone diameter 10-20 mm means strong inhibitory, clear zone diameter 5-10 mm meaning that the inhibitory power is, the clear zone diameter of 2-5 mm means weak inhibitory power. The data obtained were analyzed using the Independent-Samples T-Test Test.

RESULTS AND DISCUSSION

Descriptive data analysis of inhibition zones

Inhibitory zone data of each group were analyzed descriptively to get the mean picture, standard deviation (SD), minimum value and the maximum value obtained from the research result in table 1.

Table 1: Results of descriptive analysis of inhibitory zone data between control groups (Amoxicillin 30 µg (CT0223B)) and treatment group (Extract of Garlic (*Allium sativum*) Concentration 50%)

Group	N	Mean(mm)	SD	Max value	Min value
Control	16	32.50	1.46	35.00	30.00
Treatment	16	15.18	1.93	18.00	12.00

Analysis of the treatment effect on the inhibit zone was analyzed based on the mean of the inhibit zone between groups after treatment. Analysis of significance using Independent test t is presented in table 2.

Table 2: Mean flow analysis of inhibitory zone between control groups (Amoxicillin 30 µg (CT0223B)) and treatment group (Extract of garlic (*Allium sativum*) concentration 50%) after treatment

Group	n	Mean inhibitory zone (mm)	SD	*T*	*ρ*
Control	16	32.50	1.46	28.52	0.0001
Treatment	16	15.18	1.93		

Results of significance analysis with the independent test of the inhibitory zone after the treatment in table 2 showed that the average of inhibit zone in the two groups after treatment was significantly different (ρ<0.05). There was a difference of inhibition zone between the control group (Amoxicillin 30 µg (CT0223B)) and the treatment group (garlic extract (*Allium sativum*) 50% concentration) where the control group's inhibitory zone (Amoxicillin 30 µg (CT0223B)) was greater than the treatment group (garlic extract (*Allium sativum*) concentration 50%).

Preliminary results showed no inhibition zone in garlic extract (*Allium sativum*) concentration 5%, 20%, 35% and negative control. In garlic extract (*Allium sativum*) 50% concentration and positive control contain inhibit zone, so research used 50% concentration to know garlic extract inhibition to *Staphylococcus aureus* bacteria *in vitro*.

Based on preliminary results, researchers used a concentration of 50% as an effective concentration in inhibiting *Staphylococcus aureus* bacteria. Table 1 shows the two samples in the study were 16 repetitions to obtain a positive control zone (Amoxicillin 30 µg (CT0223B)) of 32.50 mm and 15.18 mm in the extract of garlic (*Allium sativum*). According to the classification of Davis and Stout, the two samples were classified as having a strong inhibitory effect,

but positive control (Amoxicillin 30 µg (CT0223B)) had greater inhibitory power than garlic extract (*Allium sativum*) in inhibiting the accumulation of *Staphylococcus aureus* bacteria. This is because the gram-positive bacteria have a low lipid content of only 1-4% when compared with gram-negative bacteria. Gram-positive bacteria have only one thick peptidoglycan membrane [14]. Because of this cause, the growth of *Staphylococcus aureus* bacteria can be inhibited by the garlic (*Allium sativum*) extraction containing antibacterial.

After the normality and homogeneity test, the Independent-Samples T-Test was performed to analyze the data statistically. In table 4 the results obtained that the average inhibitory zone in both groups after treatment was given significantly different ($\rho<0.05$). There was a difference of inhibition zone between the control group (Amoxicillin 30 µg (CT0223B)) and the treatment group (garlic extract (*Allium sativum*) 50% concentration) where the control group's inhibitory zone (Amoxicillin 30 µg (CT0223B)) was greater than the treatment group (garlic extract (*Allium sativum*) concentration 50%). So it can be concluded that the data test results conducted research is significant.

Garlic (*Allium sativum*) contains active ingredients, such as sativine, allicin, allyl sulphide, allyl propyl disulphide, allyl vinyl suphoxide, allistatin, Garlicin, and alkyl thiosulphinate [15]. One of the chemical material that has properties as antibacterial is allicin and allin [16]. The chemical constituents present in garlic (*Allium sativum*) is allicsin who played a role in the aroma of garlic and also kill gram-negative and gram-positive because it has a cluster of amino acids, amino benzoic, and scordinin which is a complex compound thioglosidan that serves as an antioxidant [17]. This suggests that structural differences in bacterial strains also play a role in susceptibility to the inhibitory power of garlic extract (*Allium sativum*). *Staphylococcus aureus* cell membranes contain only 2% lipids affecting the permeability of garlic extract (*Allium sativum*) [18].

This is in line with studies conducted in other studies, it has been proven that if the allicin substance is removed, will have an impact on the disappearance of antibacterial activity of garlic. It also proves that different types of garlic have different allicin content in inhibiting bacterial growth [19]. Based on research that garlic (*Allium sativum*) has a content of which is called allicin, an active substance that has antibacterial activity and has been reported to have antibacterial activity greatly to *Staphylococcus aureus* in concentrations of garlic extract 60% [20, 21].

The results can also be different because there is no standardization of extracts of natural materials so that when done in the manufacture of extracts in different laboratories, different results occur. In addition, the existence of biological variations, for example, the garlic's origin, can also affect the amount of active ingredient content available. Cultivation techniques also have a very important role in producing the maximal quality and quantity of garlic. Different cultivation techniques in each region such as planting, climates, and different treatments produce garlic with different strokes and circumstances. Another factor that may affect this research is the length of storage of extracts. The longer the extract is stored, the extract sensitivity will usually decrease. For a clinical application, this study still requires further research on the standardization of what active ingredients can be used and how effective concentrations are as antibacterial.

CONCLUSION

Based on research on the inhibition of garlic extract (*Allium sativum*) that has been done, it can be concluded that there is inhibition power of garlic extract (*Allium sativum*) concentration 50% against *Staphylococcus aureus* bacteria *in vitro*.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

There are no conflict of interest in this study

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