
COMPARISON OF THE ANTIMICROBIAL EFFECTIVENESS OF GARLIC EXTRACT AND THE ANTIBIOTIC CLINDAMYCIN ON THE GROWTH OF THE BACTERIA *PROPIONIBACTERIUM ACNES* AND *STAPHYLOCOCCUS EPIDERMIDIS***Imam Prabowo^{*1}, Daniel Natanael Malona², Oktania Sandra Puspita³**¹Pharmacy Program, Faculty of Medicine, UPN "Veteran" Jakarta, Jakarta Selatan, Indonesia²Medicine Program, Faculty of Medicine, UPN "Veteran" Jakarta, Jakarta Selatan, Indonesia³Department of Microbiology, Faculty of Medicine, UPN "Veteran" Jakarta, Jakarta Selatan, Indonesia*Correspondence: imam.prabowo@upnvj.ac.id

ABSTRACT

Acne vulgaris is an inflammatory disorder of pilosebaceous glands caused by an increase in sebum production, abnormal follicle keratinization, and colonization of positive gram *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria. Clindamycin antibiotic resistance has been reported because it is used to bacteria that cause acne vulgaris. Garlic extract has antimicrobial properties called allicin. This study aimed to determine the in vitro antimicrobial effectiveness of garlic extract in the growth of *P. acnes* and *S. epidermidis*. The samples of this study are garlic extract. This study was a true experimental study conducted using disc diffusion method with Mueller Hinton Agar. The zone of inhibition was observed on all groups of *P. acnes* and *S. epidermidis* in all tested concentrations with allicin concentration of 256 µg/ml showing the widest zone of inhibition. Statistical analysis using the One- Way ANOVA resulted in (p)<0.005. Conclusively, garlic extract was effective in inhibiting the growth of *P. acnes* and *S. epidermidis*.

Keywords: Garlic extract, *Staphylococcus epidermidis*, *Propionibacterium acnes*, antimicrobial

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INTRODUCTION

Acne vulgaris is an inflammatory disease of the pilosebaceous glands located on the face, neck, chest and back caused by increased sebum production, abnormal follicular keratinization, colonization of the anaerobic gram-positive bacteria *Propionibacterium acnes*, and local inflammation. *Propionibacterium acnes* produces extracellular products, namely lipase, protease, hyaluronidases, and chemotactic factors which cause inflammation of the skin (WHO, 2017). An increase in normal flora, namely *Staphylococcus epidermidis*, also influences the formation of acne vulgaris lesions (Dreno et. al, 2017). *Staphylococcus epidermidis* produces delta toxin which causes damage to cell membranes and causes an inflammatory response in the skin layer which results in the formation of acne vulgaris

lesions (Murray et. al 2016). Acne vulgaris is the eighth most common disease in the world. About 9.4% of people suffer from this disease. The highest prevalence occurs during prepuberty and male gender (Tan, 2015). Acne vulgaris disease in Indonesia is around 85% and occurs at the age of 14 - 17 years in women and 16 - 19 years in men (Tjekyan, 2008). Acne vulgaris can cause pain on the face, irreversible scars, systemic symptoms such as fever, and the impact of psychology on a person (Morris-Jones 2014).

The recommended treatment for acne vulgaris is the administration of topical antibiotics such as clindamycin or erythromycin. The antibiotic clindamycin is recommended for gram-positive and anaerobic bacteria (Murray et. al 2016, p. 168). Research conducted by Chandani et. al (2018) found that clindamycin is the most

frequently used topical antibiotic choice for the treatment of acne vulgaris. Clindamycin is a macrolide because it has a macrocyclic chemical compound structure that is bacteriostatic and can be bactericidal depending on the drug concentration, area of infection and organism. This antibiotic inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit, thereby preventing the organism's peptide bond formation (Bambeke, F.V., et al, 2010).

Topical administration of antibiotics increases antibiotic resistance in bacteria in the skin location and oral administration of antibiotics increases antibiotic resistance in bacteria in the human body. Alternatives to reduce the use of antibiotics in the management of acne vulgaris are needed so as not to increase antibiotic resistance in bacteria (Santer et. al, 2018).

Garlic (*Allium sativum*) has antimicrobial activity because it contains the non-protein amino acid compound alliin (Lawal et. al, 2016). Alliin is hydrolyzed by the enzyme alliinase into dehydroalanine and allylsulfenic acid when garlic is cut. The two molecules then condense spontaneously to form one allicin molecule (Rahman et. al, 2012). Allicin in garlic has antimicrobial activity by diffusing into bacterial cells and disrupting bacterial protein synthesis. This antimicrobial activity is also active on bacteria that are resistant to antibiotics (Borlinghaus et. al, 2014). Majewski (2014) in his research found that crushed fresh garlic contains allicin. Chong et. al (2014) extracted garlic using a modified maceration technique by mixing the ingredients and solvent with a blender for 1 minute and centrifuging at a speed of 7000rpm for 10 minutes to obtain an allicin concentration of $62,532 \times 10^{-1} \mu\text{g/ml}$. This research used pyrogen-free water as a solvent to ensure that there was no contamination from other bacteria and that allicin could dissolve optimally because the structure of allicin is strongly bound to water molecules. Research conducted by Saxena et. al (2018) found that the antimicrobial activity of garlic

tested using the Kirby Bauer disc diffusion technique was stronger on gram-positive bacteria compared to gram-negative bacteria. Marchese et. al (2016) found that the concentration of allicin from garlic extract which had the best antimicrobial effectiveness on *Staphylococcus epidermidis* was at an allicin concentration of $256 \mu\text{g/ml}$ and the concentration that had the weakest antimicrobial power was an allicin concentration of $32 \mu\text{g/ml}$.

MATERIALS AND METHODS

a. Method

This research uses an experimental study with a true experiment design. The research was carried out by testing the antimicrobial effectiveness of garlic (*Allium sativum*) on *Propionibacterium acnes* and *Staphylococcus epidermidis* as an experimental group and then compared with a control group as a comparison. The antimicrobial effectiveness of garlic on *Propionibacterium acnes* and *Staphylococcus epidermidis* was assessed with allicin concentrations of $512 \mu\text{g/ml}$, $256 \mu\text{g/ml}$, $128 \mu\text{g/ml}$, and $64 \mu\text{g/ml}$ obtained from extracting garlic using a modified maceration method. Antimicrobial effectiveness was observed from the zone of inhibition which was then measured using a caliper.

b. Sample

The sample used was garlic that had been extracted. Garlic is purchased at traditional markets. Garlic is washed clean, peeled, and put into a blender. The extraction process will be carried out using the maceration method with modifications by mixing 100g of garlic with 200ml of cold sterile Water for Injection (WFI) with a blender for 1 minute. Centrifuge the mixture at a speed of 7000 rpm within 10 minutes. The results of garlic extraction produced $62,532 \times 10^{-1} \mu\text{g/ml}$. The test solution used was garlic using sterile Water for Injection (WFI) solvent.

c. Sample Quantity

The sample size calculation uses the Federer formula. The treatment groups

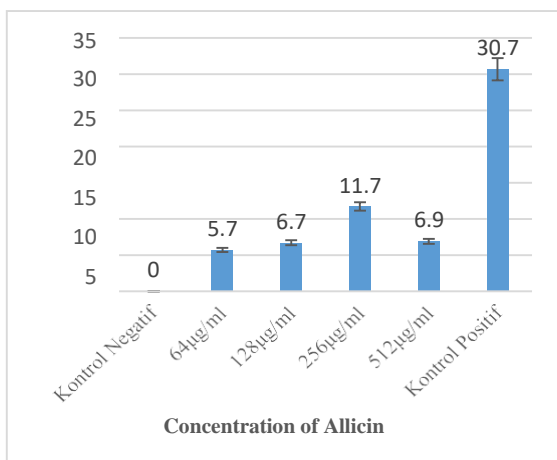
consisted of four variations of alliin concentration (512µg/ml, 256µg/ml, 128µg/ml, and 64µg/ml) with WFI solvent, one WFI negative control group, and one positive control group (clindamycin antibiotic). The minimum number of samples required for each bacterial culture based on calculations is 4 preparations of Mueller Hinton Agar (MHA) on which the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* have been cultured.

d. Procedure

The method used is the disc diffusion method. This method uses filter paper discs that have been given garlic that has been dissolved using WFI to obtain different concentrations. The filter paper is placed on the surface of a solid medium that has been inoculated with test bacteria on its surface. The solid medium that had been placed on filter paper was then incubated using an incubator for 24 hours at a temperature of 370 C. The inhibition zone formed around the disc paper was then measured to determine the antimicrobial activity against the test organisms. (American Society of Microbiology, 2010)

RESULT

Graph 1. Results of measuring the diameter of the inhibition zone formed by garlic extract against *P. acnes*



(Primary Data, 2019)

Graph 1 shows that an inhibition zone was formed in each alliin concentration treatment. This shows that the concentration of alliin contained in garlic extract has antimicrobial power so it can inhibit the growth of *P. acnes*. The optimum inhibition zone is found at an alliin concentration of 256µg/ml. The positive control group had a wider zone of inhibition compared to the group treated with garlic extract. In the negative control group, no inhibition zone was found, which means there was no inhibition of the growth of *Propionibacterium acnes*.

Table 1. Diameter of the Inhibitory Zone of *Propionibacterium acnes* by Garlic Extract.

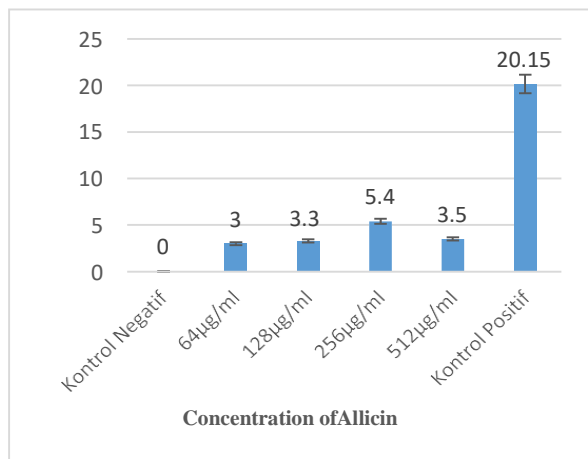
Replicat ion	Concentration (µg/ml)					
	64	128	256	512	+	-
1	1,2	1,5	4,1	3,9	31,9	0
2	13,8	14,8	18,6	15,7	30,7	0
3	2,6	3,2	15,3	2,5	30,5	0
4	5,4	7,4	9,1	5,8	29,7	0
Aver age	5,7	6,7	11,35	6,9	30,7	0
SD ±	5,64	5,92	6,74	5,97	0,9	0

Table 1 shows that the group that had the largest inhibitory zone diameter was the positive control group with an average inhibitory zone diameter of 30.7 mm, followed by the alliin concentration group of 256µg/ml with an average inhibitory zone diameter of 11.7 mm, 512µg/ml with the average inhibitory zone diameter is 6.9 mm, 128µg/ml with an average inhibitory zone diameter of 6.7 mm, and the smallest is 64µg/ml with an average inhibitory zone diameter of 5.7 mm. In the negative control group, no inhibition zone diameter was found.

The standard deviation of the research conducted shows the diversity or variation of the data obtained. This can happen because the research did not use Laminar Air Flow, so there was diversity in the data obtained (McDade et. al 1968). The use of oxygen absorbers in the research influenced the diversity of results obtained. The oxygen absorber requires time to work optimally, this results in unstable anaerobic conditions in the anaerobic jar and causes differences in bacterial growth (Charles et. al 2006)

Based on research conducted by Davis (1971) regarding the strength of antimicrobial power, treatment of groups using garlic extract with allicin concentrations of 512µg/ml, 128µg/ml, and 64µg/ml had moderate antimicrobial power, allicin concentration of 256µg/ml had antimicrobial power. strong, and the positive control has very strong antimicrobial power.

Graph 2 .Results of measuring the diameter of the inhibition zone formed by garlic extract against *S. epidermidis*.



Graph 2 shows that there is an inhibition zone formed by administering garlic extract with allicin concentrations of 512µg/ml, 256µg/ml, 128µg/ml, and 64µg/ml. This proves that garlic extract with these concentrations has antibacterial effectiveness in inhibiting bacterial growth. *Staphylococcus epidermidis*. The optimum inhibition zone was

formed when garlic extract with an allicin concentration of 256µg/ml was given. The negative control group did not have the ability to inhibit the growth of *Staphylococcus epidermidis* bacteria. In the garlic extract treatment group, the effectiveness of the inhibition zone formed was very far when compared to the positive control, namely the antibiotic clindamycin.

Table 2. Diameter of *S. epidermidis* Inhibition Zone by Garlic Extract

Replicat ion	Concentration (µg/ml)					
	64	128	256	512	+	-
1	2,5	5,0	4,3	4,8	13,8	0
2	3,3	7,5	12,8	2,5	14,6	0
3	2,0	2,9	9,7	4,7	13,8	0
4	2,1	3,5	8,5	2,0	13,0	0
Average	2,4	4,7	8,8	3,5	13,8	0
SD ±	0,59	2,05	3,51	1,45	0,65	0

Table 2 shows the average value and standard deviation of the diameter of the inhibition zone formed in the *S. epidermidis* group. The positive control group formed the largest inhibitory zone diameter with an average inhibitory zone diameter of 13.8 mm. The average zone of inhibition formed by the allicin concentration group, 256µg/ml was 8.8 mm, 128µg/ml was 4.7 mm, and 512µg/ml was 3.5 mm. The zone of inhibition in the negative control was not formed.

The standard deviation obtained shows the diversity or variation of the data. This happened because the research did not use Laminar Air Flow so there was diversity in the data obtained (McDade et. al 1968).

Davis (1971) researched the antimicrobial power of a compound to form an inhibition zone. The antimicrobial power of the group using garlic extract with an

allicin concentration of 256 μ g/ml was moderate. Allicin concentrations of 512 μ g/ml, 128 μ g/ml, and 64 μ g/ml have weak antimicrobial power.

DISCUSSION

The most effective concentration of garlic extract on *Propionibacterium acnes* and *Staphylococcus epidermidis* is at a concentration of 256 μ g/ml. The One-way ANOVA test on the *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria groups showed a significance value of <0.05, which means there was a significant difference in the treatment results between the garlic extract allicin concentration groups.

The diameter of the inhibition zone formed shows the antimicrobial power of garlic extract. The antimicrobial power of garlic extract is obtained from the non-protein amino acid compound alliin which hydrolyzes and condenses spontaneously when garlic is crushed and forms allicin. Allicin inhibits bacterial growth by diffusing into bacterial cells and reacting with cysteine residues of protein structures, resulting in failure of protein formation (Borlinghaus et. al, 2014). A high concentration of allicin indicates a high level of antimicrobial compounds which can be seen from the widening diameter of the inhibition zone as the allicin concentration increases (Nabatanzi, 2018). Garlic extract is best used 6 hours after extraction, after that the quality of the extract to inhibit bacterial growth will decrease (Astal, 2004).

The allicin concentration of garlic extract is dissolved using Water For Injection solvent. The reason for using this solvent is because the solvent is sterile and reacts neutrally to the solute, thereby reducing bias in the research. The large standard deviation in the study was caused by two factors, namely the study did not use Laminar Air Flow and an inappropriate oxygen absorber. Research conducted by McDade et. al (1968) showed that the use of Laminar Air Flow for microbiological tests can reduce bacterial contamination through particles, so that the

microbiological test data obtained is more accurate. The oxygen absorber is used to achieve anaerobic conditions in the anaerobic jar so that anaerobic bacteria can grow optimally. Oxygen absorbers require an average of 18 – 26 hours to function optimally (Charles et. al 2006). The oxygen absorber should be placed near each petri dish so that the bacterial growth in each petri dish is the same.

Research conducted by Damayanti (2014) stated that allicin is able to inhibit the growth of *Propionibacterium acnes*, but the concentration of allicin needed is not known. The research that has been carried out is in accordance with research conducted by Marchese et. al (2016) which states that an allicin concentration of 256 μ g/ml is the most effective concentration in inhibiting the growth of gram-positive bacteria, especially *Staphylococcus epidermidis* bacteria. According to the results of the research obtained, a concentration of 256 μ g/ml is also the most effective concentration for inhibiting the growth of *Propionibacterium acnes*. Increasing the concentration of garlic extract allicin on the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* to 512 μ g/ml showed a decrease in the diameter of the inhibition zone formed. This occurs because at an allicin concentration of 512 μ g/ml the diffusion of allicin into bacterial cells through the cell membrane is not optimal (Borlinghaus et. al 2014). Inadequate allicin diffusion is caused by trapping of allicin as a result of reacting with proteins and fatty acids in the plasma membrane before diffusion. Molecular density also reduces the ability to diffuse into bacterial cells (Marchese et. al, 2016).

The inhibition zone formed in the growth of *Staphylococcus epidermidis* is smaller than the inhibition zone formed in the growth of *Propionibacterium acnes*. This happens because *Staphylococcus epidermidis* produces toxins and mucus, thereby blocking phagocytosis or diffusion of antimicrobial compounds (Sinaga, 2004).

CONCLUSION

Based on the results of the research and discussion, it can be concluded that garlic extract has a strong antimicrobial effect against *Propionibacterium acnes* bacteria and a moderate antimicrobial effect against *Staphylococcus epidermidis* bacteria in vitro. the growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria, and the most effective concentration of garlic extract allicin in inhibiting the growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria was at a concentration of 256µg/ml.

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