



Antibacterial Effects of Garlic Ethyl Acetate Fraction (*Allium Sativum L.*) on the Growth of Bacteria *Staphylococcus aureus* and *Escherichia coli*

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Article Info	Abstract
<p>Article history: Received 22 May 2021 Revised 17 July 2022 Accepted 17 August 2022 Available online 01 August 2023</p> <p>Keywords: ethyl acetate fraction; garlic; <i>Staphylococcus aureus</i>; <i>Escherichia coli</i>.</p> <p>Correspondence: lmd123@ums.ac.id</p> <p>How to cite this article: Subroto LJN, Dewi LM, Sintowati R, Bestari RS. Antibacterial Effects of Garlic Ethyl Acetate Fraction (<i>Allium Sativum L.</i>) on the Growth Of Bacteria <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>. MAGNA MEDIKA Berk Ilm Kedokt dan Kesehat. 2023;10(2): 163-169</p>	<p>Background: Antibiotics are a treatment used to treat bacterial infections. However, the many antibiotic-resistant bacteria caused researchers to use plants as an alternative treatment, one of which is garlic (<i>Allium sativum L.</i>).</p> <p>Objective: Previous research stated that the diameter of the inhibition zone by garlic had a more excellent value than the antibiotics used. Hence, the researchers were interested in conducting an antibacterial test of the ethyl acetate fraction of garlic against the growth of <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>.</p> <p>Methods: This study was an experimental laboratory using the post-test-only control group design method in the Kruskal Wallis test data analysis.</p> <p>Results: the results of the inhibition analysis with p-value < 0.05 in each test group for both <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>. With the Mann-Whitney test, the results obtained a p-value < 0.005 for each bacteria and test group. The most significant value, namely p= 0.002. This result shows significant differences between the test groups for each bacterium.</p> <p>Conclusion: There is an antibacterial effect of the ethyl acetate fraction of garlic (<i>Allium sativum L.</i>) at concentrations of 25%, 50%, and 100% against the growth of <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>.</p>
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INTRODUCTION

Staphylococcus aureus is a normal flora on human skin, but it can become a pathogen in certain conditions. *Staphylococcus aureus* is also one of the bacteria with a high level of antibiotic resistance, especially the penicillin group, compared to other gram-positive bacteria^{1,2,3}. On the other side, *Escherichia coli* has a similar condition in antibiotic resistance. It is a normal flora in the human digestive tract and is one of the extra-intestinal pathogens that cause urinary tract infections, meningitis, and septicemia. In several studies, *E. coli* has been declared resistant to antibiotics, including ceftriaxone (15.7%) and levofloxacin (3.8%)⁴.

The treatment used to treat bacterial infections is antibiotics. However, in several studies, it was stated that there were cases of resistance to antibiotics^{5,6,7}. About 40-62% of them are caused by inappropriate use of antibiotics. The World Health Organization (WHO) stated that there were 2,049,442 cases of illness due to antibiotic resistance, and at least 23,000 died⁸.

Many bacteria are resistant to antibiotics. It is triggering some researchers to use plant-based material as an alternative: garlic (*Allium sativum* L.)⁸. Garlic contains a special ingredient not existing in other plants, namely Allicin. Allicin may affect bacterial growth by partially inhibiting the synthesis of RNA (Ribonucleic Acid) and bacterial protein so that it can be used as an antibacterial alternative^{10,11}.

Bakht *et al.* showed that Allicin contained in garlic was soluble in semi-polar solvents such as ethyl acetate and had a minimum bactericidal concentration (MBC) against *Staphylococcus aureus* of 330 mg/mL. Fractionation using ethyl acetate solvent is also considered more

sensitive than other solvents. The ethyl acetate fraction was stated as the most active fraction. The ethyl acetate fraction has a minimum inhibitory concentration (MIC) of 0.312 mg/mL against bacteria, meaning that at a concentration of 0.312 mg/mL, the ethyl acetate fraction has antibacterial activity¹².

In addition, Abiy & Berhe said that garlic had a larger halo zone than other antibiotics used in studying *Staphylococcus aureus* and *Escherichia coli*. Based on those data, it is encouraging to research the antibacterial effectiveness of the ethyl acetate fraction of garlic (*Allium sativum* L.) against the growth of *Staphylococcus aureus* and *Escherichia coli*⁸.

METHODS

This study was in experimental laboratory research with a post-test-only controlled group design. Here, we treated *Staphylococcus aureus* and *Escherichia coli* with the ethyl acetate fraction of garlic (*Allium sativum* L.).

The ethyl acetate fraction of garlic was prepared by dissolving the ethanolic extract of garlic made using the maceration method using 96% ethanol solvent, then dissolved using ethyl acetate solvent to dissolve the antibacterial compound contained in garlic, namely Allicin. Concentration determination of the test solution using the Geometric Progression formula according to Murtisiwi and Lindawati¹⁴

The antibacterial activity test was carried out by the well method. The inhibition zone (halo zone) was measured to assess the antibacterial activity of the ethyl acetate fraction of garlic.

The research subjects were colonies of *Staphylococcus aureus* and *Escherichia coli* obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Surakarta. Bacteria were cultured on a Mueller-Hinton medium. Samples were divided into six groups, 3 of them were the treatment groups, 1 group as a positive control, 1 group as a negative control, and 1 group as a backup. The treatment group contained garlic fractions with concentrations of 25%, 50%, and 100%. The positive control was ciprofloxacin and cefazolin, while the negative control was 1% CMC¹⁵.

Initial analysis using the Saphiro-Wilk normality test and the Levene homogeneity test. Further analysis using the non-parametric Kruskal Wallis test followed by the Post hoc test with Mann Whitney method. Ethical clearance is carried out at the Health Research Ethics Commission of the Faculty of Medicine, Universitas Muhammadiyah Surakarta, with the number 3306/A.1/KEPK-FKUMS/I/2021.

RESULTS

The investigation findings on the diameter of the inhibitory zone of each bacteria are given in Tables 1 and 2.

From Table 1, the average value of the highest inhibition zone was in the positive control group, namely cefazolin, with a value of 54.3 ± 0.81 . While in the treatment group, the largest average diameter of the inhibitory zone of the ethyl acetate fraction of garlic against *Staphylococcus aureus* was at a concentration of 100% with a value of 18.45 ± 3.32 .

From Table 2, the highest mean inhibition zone value was in the positive control group, namely ciprofloxacin, with a value of 37.1 ± 0.93 . Meanwhile, in the treatment group, the average diameter of the inhibitory zone of the ethyl acetate fraction of garlic against *Escherichia coli* was 15.251.37 at a concentration of 100%.

Table 1. Mean Diameter of Inhibition Zone of Ethyl Acetate Fraction of Garlic (*Allium sativum* L.) against *Staphylococcus Aureus*

Replication	Negative control (mm)	Positive control (mm)	The concentration of Ethyl Acetate Fraction of Garlic		
			25% (mm)	50% (mm)	100% (mm)
1	0	54	15	20	25
2	0	55	12,5	17,5	18,5
3	0	55	15,3	16,5	17,2
4	0	54	14	15	17,5
5	0	53	14	14,5	16,5
6	0	55	13,5	15	16
Mean±SD	0,00±0,00	54,3 ±0,81	14,0±1,01	16,41±2,08	18,45±3,32

Table 2. Mean Diameter of Inhibition Zone of Ethyl Acetate Fraction of Garlic (*Allium sativum* L.) against *Escherichia coli*

Replication	Negative control (mm)	Positive control (mm)	The concentration of Ethyl Acetate Fraction of Garlic		
			25% (mm)	50% (mm)	100% (mm)
1	0	37	12,5	15	17,5
2	0	36	11,5	12	13,5
3	0	38	11	12,5	15
4	0	38	12	13	16
5	0	38	11	12,5	15
6	0	36	11	13,5	14,5
Mean±SD	0,00±0,00	37,1±0,93	11,5±0,63	13,08±1,07	15,25±1,37

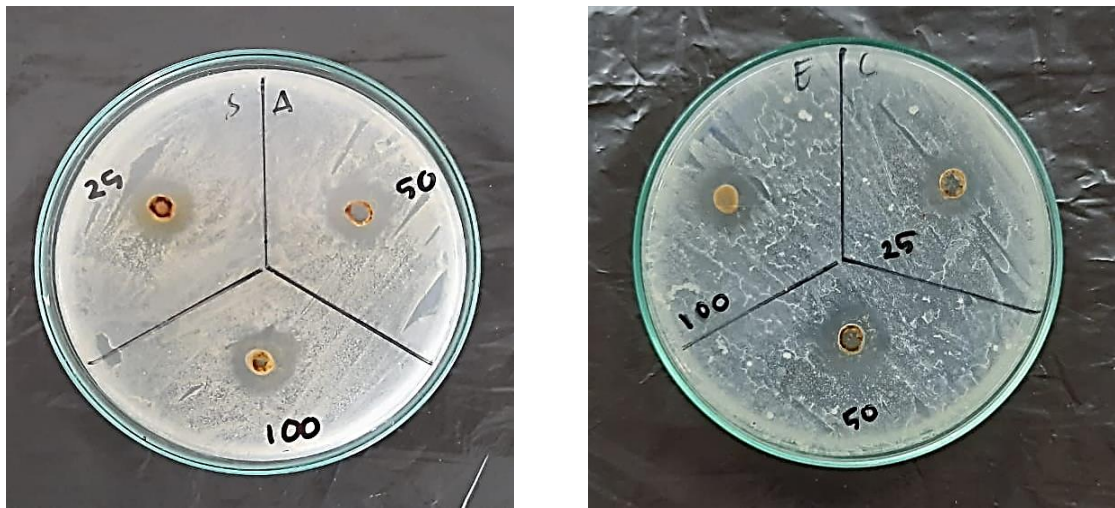


Figure 1. The inhibition zone of the ethyl acetate fraction of garlic against *Staphylococcus aureus* (left) and *Escherichia coli* (right)

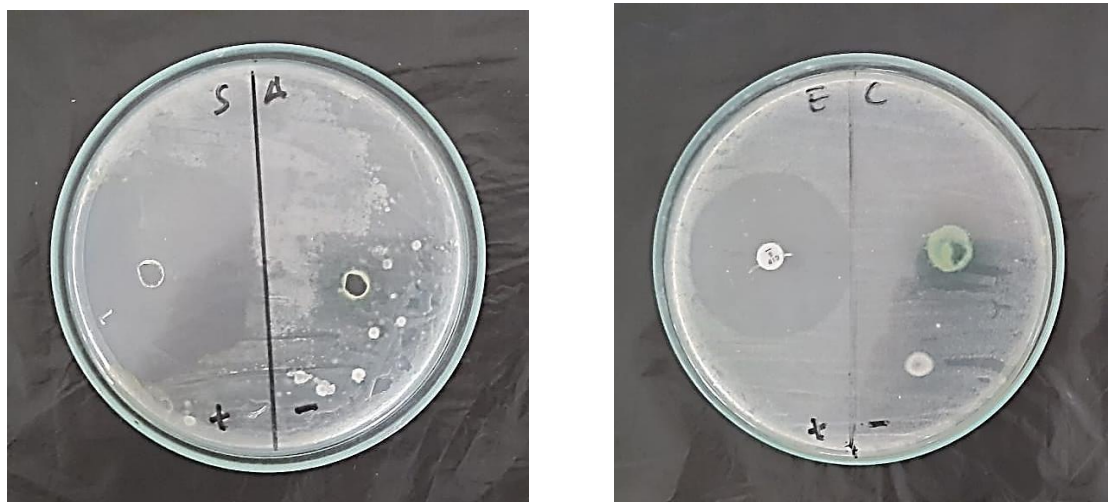


Figure 2. The inhibition zone of positive control and negative control against *Staphylococcus aureus* (left) and *Escherichia coli* (right)

DISCUSSION

Based on the data analysis that has been carried out, the results of the inhibition zone against *Staphylococcus aureus* and *Escherichia coli* have a p-value <0.05 . These results show that each bacterium significantly differs in the mean diameter of the inhibition zone in each treatment group.

The posthoc analysis of the inhibition zone found that each test group of each bacterium, *Staphylococcus aureus*, and *Escherichia coli*, had a significant value. This result can be seen from the p-value in each test group with a p-value <0.05 . The highest significance value is $p = 0.002$, and the lowest is $p = 0.036$ in *Staphylococcus aureus*.

Table 1 and Table 2 show that the positive control has a more effective inhibition zone diameter than the fraction group; it is 54.3 mm and 37.1 mm, respectively. This result indicated that the positive controls used, namely cefazolin and ciprofloxacin, had the most potent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Conversely, the negative control group, 1% CMC, has no inhibition zone. This result indicated that the negative control did not have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

Table 1 shows the results of the series test of garlic ethyl acetate fraction at concentrations of 25%, 50%, and 100% against *Staphylococcus aureus*. The average inhibition zone formed is 14.0 ± 1.01 mm, 16.41 ± 2.08 mm, and 18.45 ± 3.32 mm, respectively. This result indicates that the increasing concentration of the ethyl acetate fraction of garlic, the greater the

inhibition zone formed. The most effective garlic ethyl acetate fraction in inhibiting the growth of *Staphylococcus aureus* was at a concentration of 100%.

Table 2 shows the results of the series test of garlic ethyl acetate fraction at concentrations of 25%, 50%, and 100% against *Escherichia coli*. The average inhibition zone formed is 11.5 ± 0.63 mm, 13.08 ± 1.07 mm, and 15.25 ± 1.37 mm, respectively. This result indicates that the higher the concentration of the ethyl acetate fraction of garlic, the greater the inhibition zone formed. The ethyl acetate fraction of garlic was most effective in inhibiting *Escherichia coli* growth at a concentration of 100%.

Garlic ethyl acetate fraction with concentrations of 25%, 50%, and 100% had a higher bacterial growth inhibition value than the negative control, namely 1% CMC because 1% CMC did not have an antibacterial effect, so it could not inhibit bacterial growth. Meanwhile, compared with positive controls, namely cefazoline and ciprofloxacin, the three concentrations of the ethyl acetate fraction of garlic had lower inhibitory values. This result is not in line with the research of Abiy & Berhe, which states that garlic has a clear zone more extensive than the antibiotics used in the study⁸.

The antibacterial activity of the ethyl acetate fraction of garlic against *Staphylococcus aureus* and *Escherichia coli* can be seen from the difference in the concentration of the fraction used. The results of the study in Tables 1 and 2 found that the increasing concentration of the ethyl acetate fraction of garlic, the higher the inhibitory value of each bacterium. This study found that the concentration of 100% had the highest inhibition compared to the

concentration of 25% and 50%. This result is in line with Leyla *et al.*, which state that the concentration of the ethyl acetate fraction of garlic affects the diffusion rate. The greater the concentration of the garlic fraction, the faster the diffusion, so the antibacterial inhibition produced is also higher¹⁶. Sutrisna stated that the higher the concentration of the fraction, the greater the penetration of anti-bacterial compounds into the interior of the bacterial cell, and then it will damage cell metabolism, which causes cell death¹⁷.

Table 1 and Table 2 found that the mean diameter of the inhibition zone of the ethyl acetate fraction of garlic was higher against *Staphylococcus aureus* than against *Escherichia coli*. The difference in inhibition could be due to the more complex cell walls of *Escherichia coli* than those of *Staphylococcus aureus*, so garlic's antibacterial compounds are more difficult to diffuse into the bacterial cell membrane.^{18,19} So, the value of the higher inhibition of *Staphylococcus aureus* was obtained. Salehi stated that differences in the structure of the bacterial wall would affect the permeability of Allicin in achieving its target. The lipid content contained in the cell wall structure of *Escherichia coli* bacteria is 20%, while the cell wall of *Staphylococcus aureus* bacteria is 2%. The lipid content in each of these bacteria affects the penetration of Allicin into the bacterial cell wall²⁰.

CONCLUSION

Garlic (*Allium sativum* L.) effectively inhibits the growth of *Staphylococcus aureus* and *Escherichia coli*. The ethyl acetate fraction of garlic at concentrations of 25%, 50%, and 100% had antibacterial activity against the growth of *Staphylococcus aureus* and *Escherichia coli* with the

highest effectiveness value at a concentration of 100%. Further research on the antibacterial effect of garlic using other fractions, such as the ethanol fraction and the chloroform fraction, is needed.

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