

## Garlic Extract (*Allium sativum* L.) Effectively Inhibits *Staphylococcus aureus* and *Escherichia coli* by Invitro Test

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**Abstract:** Infection is a disease caused by the presence of pathogenic microbes, including *Staphylococcus aureus* and *Escherichia coli*. Garlic (*Allium sativum* L.) has chemical contents such as allicin, alkaloids, flavonoids, saponins, tannins, and steroids, which can function as an antibacterial against *Staphylococcus aureus* and *Escherichia coli*. This study aims to determine the antibacterial properties of garlic extract powder against *Staphylococcus aureus* and *Escherichia coli*. This research is the initial stage of the development of herbal medicines to treat *Staphylococcus aureus* and *Escherichia coli* infections. The antibacterial activity test was carried out by the liquid dilution method. The concentrations used were 30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/mL and 70 mg/mL. The results showed that the Minimum Inhibitory Concentration (MIC) against *Staphylococcus aureus* and *Escherichia coli* was 40 mg/mL and 50 mg / mL. Minimum Bactericidal Concentration (MBC) results for *Staphylococcus aureus* and *Escherichia coli* are 50 mg/mL and 70 mg/mL. Based on the Simple Linear Regression test, the R<sup>2</sup> value of *Staphylococcus aureus* and *Escherichia coli* is 0.545 and 0.785, so it can be concluded that there is an effect of garlic extract powder on the growth of *Staphylococcus aureus* and *Escherichia coli* by 54.5% and 78.5%. Garlic (*Allium sativum* L.) extract powder has potential as herbal medicine against bacterial infections but requires further research to determine its effect in vivo.

**Keywords:** Garlic (*Allium sativum* L.); *Staphylococcus aureus*; *Escherichia coli*

### INTRODUCTION

Infection is a disease caused by pathogenic microorganisms in the body. In the human body, naturally, there are floranormal bacteria that are beneficial to the body. One example of floranormal bacteria that exist in the human body is *Staphylococcus aureus* and *Escherichia coli*. Floranormal of bacteria can turn into pathogens if the amount is more than normal levels. *Staphylococcus aureus* is a bacterium that lives as a floranormal in the nasal passages, skin, and mucous membranes in humans. The disease caused by *Staphylococcus aureus*, which often occurs, is staphylococcal scalded skin syndrome (SSSS). The death rate due to the disease in children is very low (1-5%), whereas, in adults, it is higher (50-60%)<sup>1</sup>. *Escherichia coli* is a floranormal that colonizes the intestine but does not rule out that this bacterium becomes pathogenic and causes infection. The diseases often caused by *Escherichia coli* are urinary tract infections by 90%, bacteremia, diarrhea, meningitis, and sepsis<sup>2</sup>.

These microorganisms have been resistant to the drugs given, *Staphylococcus aureus* is resistant to Methicillin-Resistant *Staphylococcus aureus* (MRSA) by 84.4%, and *Escherichia coli* is resistant to ceftazidime by 66.7%<sup>3</sup>. The effects of antibiotics

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that already exist besides the occurrence of resistance are also other effects such as ototoxic effects, nephrotoxic effects, teratogenic effects, hypersensitivity reactions, and bleeding. This situation encourages researchers to find safer alternative treatments, including medicines from natural ingredients—one of the plants that have potential as a medicinal plant is garlic. Garlic (*Allium sativum* L.) has the potential as a substitute for antibiotics and is easy to apply in powders<sup>4</sup>. The powder has more extended durability in the storage process and can intend for oral or external use. The content of chemical compounds found in garlic is allicin, alkaloids, flavonoids, saponins, tannins, and steroids<sup>5</sup>. Allicin believed to be an active ingredient that plays a role in the antibacterial effect, both gram-positive and gram-negative bacteria<sup>6</sup>. Allicin requires the right solvent to remain stable during extraction using ethanol<sup>7</sup>.

A research in 2016 shows that garlic (*Allium sativum* L.) juice can inhibit the growth of *Staphylococcus aureus* (MIC) at a concentration of 3.12% and the ability to kill (MBC) at a concentration of 6.25%<sup>8</sup>. Another study conducted in 2018 states the Minimum Bactericidal Concentration (MBC) of ethanol extract of garlic that has fermented against the growth of *Escherichia coli* obtained at a concentration of 85% and in *Staphylococcus aureus* obtained at a concentration of 90%<sup>9</sup>. Previous research has carried out on garlic (*Allium sativum* L.) in various dosage forms such as juice and ethanol extract. However, antibacterial research in garlic (*Allium sativum* L.) preparations form of dry powder has not carried out. This study aims to determine the antibacterial effectiveness of garlic extract powder (*Allium sativum* L.) on the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria.

## MATERIALS AND METHOD

The research was an experimental study with the Posttest Only Control Group Design, namely by examining MIC and MBC of garlic extract powder (*Allium sativum* L.) at concentrations of 30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/mL and 70 mg/mL against the growth of *Staphylococcus aureus* and *Escherichia coli*. Then compared to the negative control group (TSB solution), positive control (chloramphenicol) and ethanol extract control of garlic (*Allium sativum* L.) The material used in this study was garlic (*Allium sativum* L.) obtained from the Banjarbaru area, South Kalimantan, Indonesia. The part of garlic used is a good and fresh garlic bulb with the criteria to contain, not wrinkled, and the tuber does not show the black part.

The independent variable in this study was the concentration of garlic extract powder (*Allium sativum* L.), namely 30 mg / mL, 40 mg / mL, 50 mg / mL, 60 mg / mL and 70 mg / mL. This study's dependent variable was the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria based on the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Garlic (*Allium sativum* L.) tubers are peeled, washed thoroughly, then weighed and blended. The maceration process with 96% ethanol (1: 4) carried out for three days. Maceration concentrated using a water bath at 50°C until a thick extract obtained. Three grams of thick extract plus 1 gram aerosil is homogenized and allowed to stand overnight. The mixture was sieved using a 60 mesh sieve to obtain 2 g of fine powder.

The concentration of 200 mg/mL garlic extract powder made by 2 grams of garlic extract powder dissolved into 10 mL aquadest. Making a concentration of 60 mg/mL, 80 mg/mL, 100 mg/mL, 120 mg/mL and 140 mg/mL using a concentration of 200 mg / mL and aqua dest. Suspension of *Staphylococcus aureus* and *Escherichia coli* results from bacterial incubation for 24 hours at 37°C. MIC test was done by mixing 1 mL of the test solution and 1 mL of bacterial suspension with repetition three times. The final concentration of the test solution after adding the bacterial suspension is half

of the initial concentration so that the concentration of the test solution becomes 30 mg/mL, 40 mg/mL, 50 mg/mL, 60 mg/mL and 70 mg/mL. MIC results after 24-hour incubation at 37°C determined in solution by measuring absorbance at a UV-Vis spectrophotometer with a wavelength of 400 nm. Absorbance values before and after incubation compared. After incubation, increasing the absorbance value indicates the growth of living bacterial cells. In contrast, the constant and decreasing value of absorbance after incubation indicates the absence of live bacterial cell growth<sup>14</sup>. The solution showing the MIC taken 20 ul spread on the surface of the Nutrient agar incubation plate 24 hours at 37°C. MBC demonstrated with Nutrient agar plates that had no growth in bacterial colonies.

## RESULTS AND DISCUSSION

Antimicrobial testing of garlic extract powder (*Allium sativum* L.) on the growth of *Staphylococcus aureus* using the Minimum Inhibitory Concentration (MIC) test showed a decrease in absorbance at the lowest concentration of 40 mg / mL. Minimum Bactericidal Concentration (MBC) test, which is characterized by the absence of colony growth at the lowest concentration for *Staphylococcus aureus* occurs at a concentration of 50 mg/mL. The results can be seen in Table 1 below. The average number of *Staphylococcus aureus* colonies for each concentration in the minimum kill concentration test presented, as shown in Figure 1.

Table 1. Minimum Bactericidal Concentration (MBC) Results of Garlic Extract Powder for *Staphylococcus aureus* Growth

Garlic Powder Extract Concentration	Repetition					Average (Colony)
	I	II	III	IV	V	
30 mg/ml	7	11	5	13	16	10
40 mg/mL	3	2	0	3	4	2
50 mg/mL	0	0	0	0	0	0
60 mg/mL	0	0	0	0	0	0
70 mg/mL	0	0	0	0	0	0
Control (+)	0	0	0	0	0	0
Control (-)	349	361	342	377	392	364
Garlic Control	0	0	0	0	0	0

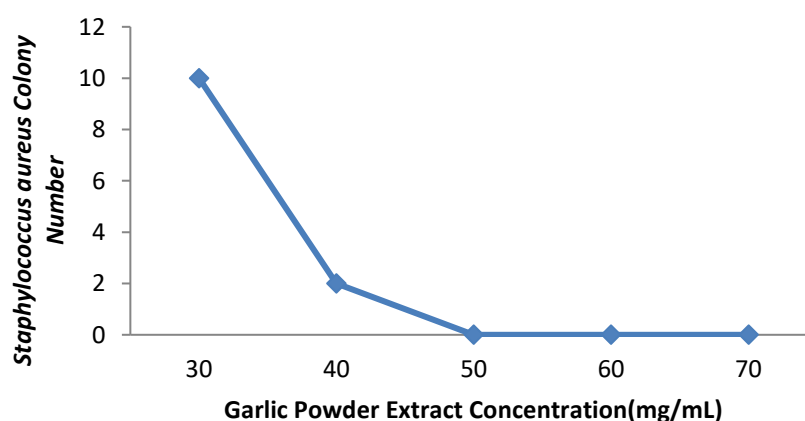


Figure 1. Graph of Average Number of *Staphylococcus aureus* Colonies in Various Concentrations in MBC

Antimicrobial testing of garlic extract powder (*Allium sativum* L.) on the growth of *Escherichia coli* using the Minimum Inhibitory Concentration (MIC) test showed a decrease in absorbance at the lowest concentration of 50 mg / mL. The Minimum Bactericidal Concentration (MBC) test marked by the absence of colony growth at the lowest concentration for *Escherichia coli* occurs at 70 mg/mL. The results can be seen in Table 2. The results of the average number of *Escherichia coli* colonies for each concentration in the minimum kill concentration test presented in Figure 2.

Table 2 Minimum Bactericidal Concentration (MBC) Results of Garlic Extract Powder for *Escherichia coli* Growth

Garlic Powder Extract Concentration	Repetition					Average (Colony)
	I	II	III	IV	V	
30 mg/MI	87	84	88	96	121	95
40 mg/mL	62	25	51	59	83	56
50 mg/mL	4	3	6	11	19	9
60 mg/mL	1	0	2	2	3	2
70 mg/mL	0	0	0	0	0	0
Control (+)	0	0	0	0	0	0
Control (-)	468	457	473	479	482	472
Garlic Control	0	0	0	0	0	0

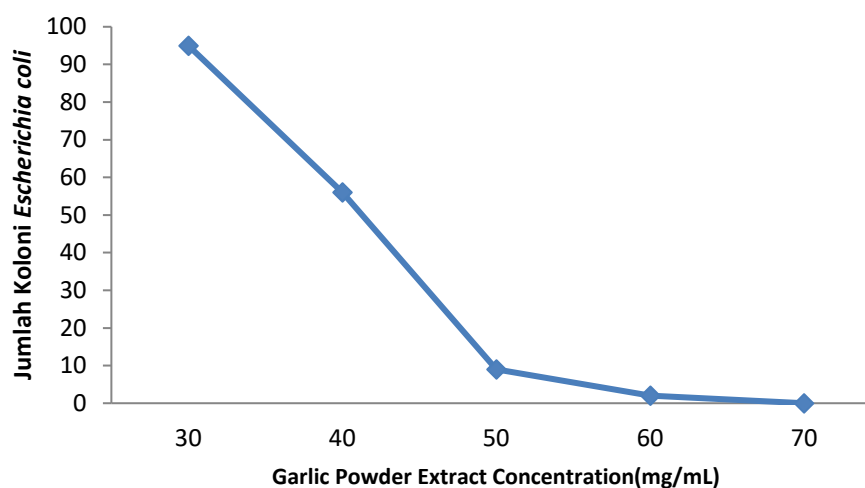


Figure 2. Graph of the Average Number of *Escherichia coli* Colonies in Different Concentrations in MBC

Garlic (*Allium sativum* L.) is one type of plant usually used as a spice to provide a distinctive savory taste in food. This study uses the garlic plant parts in the form of garlic bulbs. Garlic has active compounds such as allicin, alkaloids, flavonoids, saponins, tannins, steroids. According to Moulia et al. in 2018, there are secondary metabolites that are biologically very useful; most of these compounds contain sulfur, which is responsible for the taste, aroma, and pharmacological properties of garlic<sup>10</sup>.

This research conducted to determine the effect of garlic extract powder (*Allium sativum* L.) on the growth of *Staphylococcus aureus* and *Escherichia coli*. Garlic extract powder obtained through the extraction process by maceration using 96% ethanol solvent. Garlic crushed before macerated so that alliin can be formed because the injury to garlic causes alliin compounds to react with the alliinase enzyme to alliin<sup>5</sup>. The liquid extract obtained is an added aerosil to be a powder. Aerosil helps increase the homogeneity of the mixture and protect the material from moisture, both used for extracts that are hygroscopic<sup>11</sup>.

The study continued with determining the minimum inhibitory concentration and minimum kill concentration. In the MIC test, the reading of the results is carried out by the spectrophotometric method by comparing the absorbance values produced before and after incubation using a UV-Vis spectrophotometer. UV-Vis spectrophotometer tool serves to measure the wavelength and the value of turbidity absorbance present in each treatment<sup>8</sup>.

Based on Tables 1 and 3 from the measurement results using UV-Vis Spectrophotometer, it found that MIC of garlic extract powder against *Staphylococcus aureus* was 40 mg / mL while *Escherichia coli* at a concentration of 50 mg / mL. This concentration is the smallest concentration that has decreased absorbance value after incubation 1x24 hours, which can inhibit bacterial growth. Increased absorbance values indicate continued bacterial growth, while absorbance values that remain or decrease indicate inhibited bacterial growth<sup>8</sup>. These results differ from study in 2018 about the antibacterial effect of garlic juice (*Allium sativum* L.) on *Staphylococcus aureus* showing MIC *Staphylococcus aureus* occurs at a concentration of 100 mg/mL<sup>12</sup>. Another study by Abiy and Berhe regarding the antibacterial effect of garlic (*Allium sativum*) against clinical isolates of *Escherichia coli* shows that MIC *Escherichia coli* occurs at a concentration of 15 mg/mL<sup>13</sup>. There is a difference in the minimum inhibitory concentration range between *Staphylococcus aureus* and *Escherichia coli*, wherein this study, *Escherichia coli*, had a higher concentration range than *Staphylococcus aureus*.

The difference in the results of this study with other studies can be caused by the method of making samples, in the Sari study using garlic juice, with the juice according to Sarah et al., the active substances contained in garlic did not come out optimally, which causes active substances that dissolve only a few so that the inhibition against bacterial growth is not optimal but with the extraction process will get active starch/juice from garlic that has been separated from the water content in garlic so that the active content in garlic which is antimicrobial will there is more in garlic extract than in garlic juice. This study uses garlic powder obtained through the extraction process with ethanol as a solvent. The ethanol solvent used because it can dissolve almost all substances, both polar, semipolar, and nonpolar<sup>14</sup>. Another study by Abiy and Berhe in 2016 used soxhlet extraction techniques. Researchers used maceration techniques because the soxhlet extraction method could easily filter out more active compounds than maceration methods<sup>15</sup>.

Garlic, besides having the ability to inhibit also can kill the bacteria *Staphylococcus aureus* and *Escherichia coli*, this is indicated in the Minimum Bactericidal Concentration (MBC) test. Based on Tables 2 and 4, which are the results of growth and calculation of the number of colonies, the minimum kill concentration of *Staphylococcus aureus* is 50 mg/mL, and *Escherichia coli* is 70 mg/mL at these concentrations there is no growth of *Staphylococcus aureus* and *Escherichia coli*.

The results of this study indicate that the higher the concentration of garlic extract powder used, the greater the ability to inhibit and kill *Staphylococcus aureus*

and *Escherichia coli*. The decrease indicated this in absorbance value in Tryptic Soy Broth (TSB) media in the liquid dilution test. It decreased the number of *Staphylococcus aureus* and *Escherichia coli* colonies on Nutrient Agar (NA) media.

The concentration of garlic extract powder with different ranges in killing gram-positive bacteria such as *Staphylococcus aureus* and gram-negative bacteria such as *Escherichia coli* is because gram-negative bacteria can produce antibiotic-destroying enzymes known as the enzyme adenylase, phosphorylase, or acetylase so that it can destroy or damaging the antibacterial substances possessed by garlic<sup>16</sup>. According to El-Mahmood, the envelope of gram-negative bacteria is naturally more complex than the gram-positive bacterial envelope structure so that it complicates the process of penetration of antimicrobial agents into the walls of gram-negative bacterial cells<sup>17</sup>.

Based on the work process of antimicrobial substances in influencing bacterial growth, Komala et al. stated that the alkaloids in garlic work to disrupt the constituent components of peptidoglycan in bacterial cells that the cell wall layers are not formed intact and cause cell death<sup>18</sup>. According to Rijayanti et al., flavonoids can form complexes with bacterial extracellular proteins, so that protein denaturation occurs, and the more lipophilic a flavonoid is, the ability to damage bacterial cell walls is stronger<sup>19</sup>. Besides the mechanism of action of flavonoids as an antibacterial by damaging bacterial cell walls consisting of lipids and amino acids and damaging the structure of bacterial DNA, the cell nucleus will lysis and bacteria die<sup>18</sup>.

Tannins can inhibit the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot be formed<sup>20</sup>. Similarly, steroids can interfere with the lipid membrane function, which causes leakage of the liposomes so that the cells are brittle and lysis<sup>18</sup>. Salima's research shows that the main component responsible for inhibiting and killing bacteria is allicin<sup>21</sup>. According to Mikaili et al., Allicin shows an antibacterial effect by inhibiting lipid synthesis production. This inhibition causes amino acids and proteins to produce, and bacterial cell walls cannot be formed<sup>6</sup>.

The limitation of this study is that there was no MIC and MBC test for garlic (*Allium sativum* L.) thick extract before aerosil was added. It is possible that the MIC and MBC values in the thick garlic (*Allium sativum* L.) extract were at a smaller concentration, because without the addition of aerosil.

## CONCLUSION

Minimum Inhibitory Concentration (MIC) of garlic extract powder (*Allium sativum* L.) against *Staphylococcus aureus* occurs at a 40 mg/mL concentration. Against *Escherichia coli* occurs at a concentration of 50 mg/mL. Minimum Bactericidal Concentration (MBC) garlic extract powder (*Allium sativum* L.) against *Staphylococcus aureus* occurs at a 50 mg/mL concentration. Against *Escherichia coli* occurs at a concentration of 70 mg/mL. There is an effect of garlic extract powder (*Allium sativum* L.) on the growth of *Staphylococcus aureus* by 54.5% and *Escherichia coli* by 78.5%, which is indicated by the R<sup>2</sup> value of *Staphylococcus aureus* is 0.545, and *Escherichia coli* is 0.785 by using the Test Simple Linear Regression. Garlic (*Allium sativum* L.) extract powder has potential as herbal medicine against bacterial infections but requires further research to determine its effect in vivo.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest and have not received any funds for this study.

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