

Antibacterial Activity of Ethanolic Extract of Leaves and Bulb of *Allium sativum* Linn. against *Shigella sonnei*

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Abstract

Garlic (*Allium sativum* Linn.) is widely known by people as herb, besides having antibacterial property. Alisin is one of antibacterial active substance contained in garlic. The purpose of this study was to test the antibacterial activity of ethanol extracts of garlic leaves and bulbs against *Shigella sonnei* or dysentery bacteria. The determination of antibacterial activity was conducted by using disk diffusion and dilution methods. Antibacterial test results which had the best inhibitory activities against *Shigella sonnei* were shown by the extracts of 8% garlic bulbs and 4% garlic leaves with inhibition zone respectively 16mm and 19mm. The result showed the MIC of garlic bulbs extract was 0.5% and garlic leaves extract was 0.25%. The smallest concentration which could kill (MBC) *Shigella sonnei* was garlic bulbs extract 1% and garlic leaves extract 0.5%. This study showed that the ethanol extracts of garlic leaves and bulbs had antibacterial activity against *Shigella sonnei*.

Keywords: antibacterial activity, *Allium sativum*, *Shigella sonnei*, MIC, MBC

1. Introduction

Garlic is often known by the public as a spice in the kitchen, but it also has many properties that make it as a useful raw material for making herbal medicines. Garlic is known to help maintain normal cholesterol levels, but it has antibacterial activity against several types of bacteria. Garlic is shaped like a top, has strong-smell, is white, from familia Alliaceae, and its Latin name is *Allium sativum* Linn. Garlic contains volatile oil, diallyl sulfide, alisin, alinase enzymes, saponins, flavonoids, polyphenols, vitamins A, B, and C (Indonesia, 1995).

One of the active substance contained in garlic that functions as an antimicrobial in addition to the essential oil is alisin. Alisin can effectively kill microbes, such as bacteria causing flu infection, gastroenteritis or fever. Alisin could kill gram-positive and gram-negative bacteria (Syamsiah, 2003). The antibacterial effect of garlic is caused due to the presence of compounds such as alisin tiosulfinat; when alisin diallyl disulfide is reduced, the antibacterial activity will increase. Alisin inhibits the growth of bacteria by directly inhibiting synthesis of RNA. Even though the synthesis of DNA and protein synthesis is also inhibited, the synthesis of RNA are the prime targets of alisin (Durairaj, S., Srinivasan, S., and Lakshmanaperumalsamy, 2010).

People often experience acute diarrhea or dysentery, one of which is caused by *Shigella sonnei*. *Shigella sonnei* is a gram-negative bacteria in the family Enterobacteriaceae which often causes severe diarrhea (Qu F., Bao C., Chen S., Cui E., Guo T., Wang H., Zhang J., Wang H., 2012) and can cause dysentery (Gould, D and Brooker, 2003). Problems handling of infections

caused by *Shigella* sp. exacerbated by the emergence of resistant *Shigella* sp. to some antibiotics like ampicillin, tetracycline, trimethoprim and sulfamethoxazole. Based on the above review, research needs to be done to test the potential of ethanol extract of leaves and bulbs of garlic as an antibacterial herbal medicine against *Shigella sonnei*.

2. Materials and Methods

2.1 Tools

The research utilized rotary evaporator, water bath, petri dishes, test tubes, glass spreader, micropipette, incubators, and chamber.

2.2 Ingredients

The ingredients of the research were garlic, leeks, 96% ethanol, the culture of *Shigella sonnei*, BHI, MH (Mueller Hinton), blank disk, distilled water, DMSO, silica gel GF254 plate, ethyl acetate, and methanol.

2.3 Making Ethanol Leaf Extract and Garlic Bulbs

Maceration was done by weighing as much as 1 kg of garlic bulbs that had been peeled and cleaned and then crushed, and put in a vessel. Ninety-six percent of ethanol was added as much as 7.5 liters (1 part materials using 7.5 parts solvent), allowed to stand for 5 days while stirring often (Departemen Kesehatan RI, 1979). Maserat was obtained by filtering the mixture by using a Buchner funnel to separate the pulp, then evaporated. The evaporated mixture was heated over a water bath to obtain a thick extract of garlic bulbs. The maceration of leeks was conducted by weighing 1 kg of leeks that had been dried, then crushed and put in a vessel. The next step was similar to the steps of making the bulb of garlic extract.

2.4 Antibacterial Activity Test (Diffusion method)

MH Media inoculated with the bacteria *Shigella sonnei* 10^8 CFU/mL then the disk that had been added by each concentration of the 10 mL DMSO extract of the leaves and bulbs of garlic was placed on the media. It was incubated for 24 hours at 37°C. Furthermore, the researchers observed the presence or absence of inhibition zone in the disk. The zone of inhibition was measured by using calipers.

2.5 Antibacterial Activity Test (Dilution Method)

The next step was the dilution method. The researchers prepared 9 sterile tubes, to the tube 2 to 9 were filled by liquid BHI medium as much as 0.5 mL. The tubes 1 and 2 were filled with 0.5 mL ethanol extract of the leaves and bulbs of garlic with a concentration of 100%. Furthermore, the researchers took 0.5 mL of 2 and a tube was inserted into the tube 3, and the treatment was also done to the tube 7. The tube 7 and then disposed of 0.5 mL tube 8 was not filled with ethanol extract of the leaves and bulbs of garlic. Tubes 1 to 8 were filled with 0.5 mL suspension of bacteria *Shigella sonnei*. Tube 9 that only contained liquid BHI medium was used as control medium. Minimum Inhibitory Concentration (MIC) was characterized by clarity of the test solution which was the lowest concentration that could inhibit the growth of bacteria in BHI medium. Minimum Bactericidal Concentration (MBC) inspection was carried out by taking 50 mL of each tube and etched in MH medium and then incubated at 37 ° C for 24 hours.

2.6 TLC test

Ethanol extract of bulbs of garlic was dissolved in methanol, and then spotted on a silica TLC plate and inserted into the chamber that was already saturated with mobile phase of ethyl acetate: methanol (with ratio 1:1).

3. Result and Discussion

Garlic bulbs were tested by TLC to determine the content of alisin. Elution that was done by using a mobile phase of ethyl acetate: methanol (with ratio 1:1) could not separate the active components of the substances in garlic. The results of TLC showed that there was only one spot that did not split. It could be seen in Figure 1.



Figure 1. Results of TLC ethanolic extract of garlic bulbs

Antibacterial activity test by disk diffusion method obtained inhibitory zone diameters. Ethanol extract of each leaf and bulb of garlic made a series of concentration dissolved in dimethyl sulfoxide (DMSO). The diameter of inhibition zone with the concentration of ethanol extract in 40 mg/mL of leaves and ethanol extract in 80 mg/mL of garlic bulbs against *Shigella sonnei* were 19 ± 1.0 mm and 16 ± 2.1 mm (Figure 2). The results of inhibition zone diameter could be seen in Table 1 and the diameter of inhibition zone of combinations of the ethanol extract of the leaves and bulbs of garlic could be seen in Table 2.

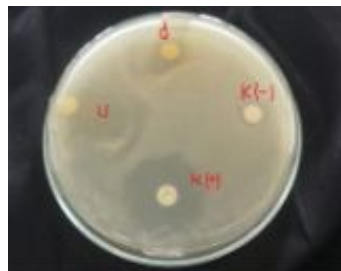


Figure 2. The diameter of inhibition zone ethanol extract of the leaves and bulbs of garlic against *Shigella sonnei*

Table 1. Inhibition zone diameter (mm) of ethanol extract of the leaves and bulbs of garlic (mg/mL) against *Shigella sonnei*

Extracts in DMSO	Extract concentration (mg/mL)	Diameter of inhibition zone (mm)
Ethanol extract of garlic bulbs	20	10±1.5 mm
	40	14±1.0 mm
	80	16±2.1 mm
	160	13±3.0 mm
	320	12±1.7 mm
Ethanol extract of garlic leaves	20	14±1.0 mm
	40	19±1.0 mm
	80	11±0.6 mm
	160	12±2.8 mm
	320	12±3.5 mm

Table 2. Diameter of inhibition zone of combinations of the ethanol extract of the leaves and bulbs of garlic

Ratio ethanol extract of garlic bulbs:garlic leaves	Extract concentration (% w/v)		Diameter of inhibition zone (mm)
	Bulbs of garlic	Leaves of garlic	
1:1	2	2	11±0.5 mm
1:2	1	2	12±1.5 mm
2:1	2	1	13±0.5 mm

The ethanol extract bulb of garlic and leeks with a ratio of 2:1 had the greatest inhibition zone, and then the antibacterial activity was tested by using the dilution method. The results of the MIC using dilution method were 0.5% ethanol extract of garlic bulbs and 0.25% ethanol extract of garlic leaves, which was shown in the tube 3 (figure 3). The MIC results obtained MBC, which was then calculated from the ethanol extract of garlic bulbs and ethanol extract of garlic leaves against *Shigella sonnei*, respectively by 1% and 0.5% obtained from the MBC tube 2 (Figure 4).

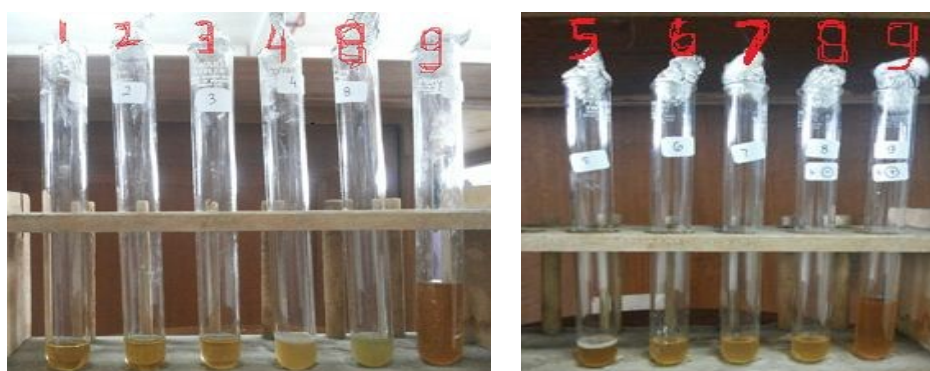


Figure 3. Results MIC ratio of the ethanol extract of garlic bulbs and ethanol extract of garlic leaves (2:1)

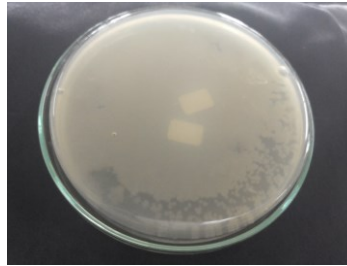


Figure 4. Results MBC ratio of the ethanol extract of garlic bulbs and ethanol extract of garlic leaves (2:1)

4. Conclusion

The research findings showed that ethanolic extract of the leaves and bulbs of garlic (*Allium sativum* Linn.) had antibacterial activity against *Shigella sonnei*.

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