

# Antibacterial Activities of *Allium Sativum* (Garlic) Extracts Against *Staphylococcus Aureus* and *Escherichia Coli*

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**Abstract:** The search for using natural product and plant compounds as antimicrobial agents has been increased in the last decades. This study aimed of evaluate the antibacterial effects of *Allium Sativum* (garlic) extracts against *Staphylococcus aureus* and *Escherichia coli*. The garlic were peel and grinded until it turned into puree form. The garlic extracts were homogenized and mixed with distilled water to obtain four dilution concentration; 25% garlic extract, 50% garlic extract, 75% garlic extract and 100% garlic extract. The extract was tested against *S. aureus* and *E. coli*. All garlic extracts concentration demonstrated varying degrees of antimicrobial activities against both of bacteria. However, the zones of inhibition obtained using 100% of garlic extract were larger compared with those of lower garlic concentrations against both *S. aureus* and *E. coli*. The results suggest that garlic extract highly active antimicrobial components. Further investigation is warranted to improve the extraction methods and to identify the antibacterial activity of garlic extracts against different bacterial species.

**Keywords:** Garlic, Antibacterial, Zone of inhibition, MIC, MBC

## 1. INTRODUCTION:

Continual development of bacterial resistance toward various antibiotics can cause potentially life-threatening infections by gram-positive and gram-negative bacteria Sathya and Shoba (2014). In addition, excessive use of antibiotics may disturb the intestinal microflora and may even enhance the transmission of deadly organisms such as methicillin-resistant *Staphylococcus aureus* (Blaser and Falkow 2009).

Usage of plants and their extracts is an ancient procedure of conventional medicine is to treat infections. Many plants have been used because of their antimicrobial therapies, which are primarily synthesised during the plants' secondary metabolism. Herbal medicine can be in the form of powders, liquid or mixtures, ointment linings and incision linings that can be rowed or boiled (Jehan *et al.*, 2011; Hassan and Himayat Ullah 2019).

*Allium sativum* Linn commonly known as garlic belongs to the *Liliaceae* family and falls within the group of onion had gained lots of attention from modern medicine as it contains more than 200 chemical substances and it is commonly used to protect human body from many diseases due to extensive beliefs of its efficacy in improving overall well-being (Bayan *et al.*, 2014). The main bioactive compound that can be found in the garlic's aqueous extract or smashed raw garlic is known as allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate). which has the role as anti-infective agents against of bacteria, parasites, and fungi (Abramovitz, D. *et al.*, 1999; Karunanidhi *et al.*, 2019). It also has protective properties for the liver, including the antitumor agents (Goncagul and Ayaz, 2009). Allicin, an

organosulfur compound, which prevents lipid biosynthesis, was proved to damage *Candida albicans* cell wall and cause inhibition of RNA synthesis in bacteria (Mahomoodally *et al.*, 2018). These findings were usually limited to a few distinct bacterial species, rather than to the systemic treatment of infection (Müller *et al.*, 2016).

Therefore, the objective of this study was to establish the effectiveness of using garlic extracts as a new source of antimicrobial agents that is effectively inhibit growth of bacteria with minimum side effects and compares their effect with the effect of some antibiotics.

## 2. MATERIALS AND METHODS

### Collection and preparation of garlic

Fresh garlics (*Allium sativum* L.) were collected from the market in Kuchai Lama, Kuala Lumpur, Malaysia. The plants were washed with tap water, followed by ethanol and distilled water then the plants were dried in an incubator. The garlic were then peeled and weighed and sliced into smaller pieces using sterile knife. The sliced garlic were grinded using an electric grinder until it turned into puree form. Thee extract was filtered using the sterile cheesecloth. This crude extract was then stored in sterile capped bottle at 4°C to 8°C for further use according to Fagbemi, *et al.* (2009).

The garlic extracts were mixed with distilled water and were assigned to one of four dilution concentration. The first concentration was obtained by mixing 2.5 ml of pure garlic extract with 7.5ml of sterile distilled water (A), second concentration by mixing 5ml of garlic extract with 5ml of sterile distilled water (B) third concentration by mixing 7.5ml of garlic extract with 2.5ml of sterile distilled water (C) and the last concentration was 10ml of garlic extract without sterile distilled water (D).

### Microorganisms

Microbial cultures were obtained from microbiology laboratory of Management and Science University (MSU). Bacteria were used in this study includes one Gram-positive bacteria *Staphylococcus aureus* (ATCC 29213) and one Gram-negative bacteria *Escherichia coli* (ATCC 25922).

### Procedure of disc diffusion method

Antimicrobial activities were assessed by the disk diffusion method in the presence of the extract. A suspension of bacterial inoculum was adjusted to a McFarland standard of 0.5 and was spread gently onto Mueller–Hinton agar plates with a cotton-tip applicator. A sterile-filter paper disks of 6 mm in diameter was prepared and impregnated with 10µl of each concentration of plant extract from the 4 different universal bottles labelled as A, B, C, and D. We use Vancomycin disc (10µl/ml) as positive control for gram positive bacteria *S. aureus* and Amikacin disc (10µl/ml) for gram negative bacteria *E. coli*. The plates were incubated for 24 hours at 37°C. T After incubation, the antimicrobial assay was determined by measuring the diameter of the zone of inhibition observed (Ulusoylu *et al.*, 2001). Each test was performed in triplicates and the average of the results were attained.

### Determination of Minimum inhibitory concentration (MIC)

The MIC of garlic extract was determined in triplicates by the microdilution broth method as described by the Clinical and Laboratory Standards Institute (2011). Two folds' serial dilution of garlic extract from various concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.195 mg/mL were poured into the 96 well microplates. Bacterial suspensions were accustomed to the logarithmic-phase growth to follow the turbidity of a 0.5 McFarland standard yielding  $1 \times 10^8$  CFU/ml (Yilmaz,2012). The bacteria were added to all the wells and the microplates were incubated for 24 hours at 37°C. After incubation, resazurin were

added to each well with and left in the incubator for minimum 30 minutes for the observation of colour change comparing to the controls. Bacterial suspension in the well filled with nutrient broth was considered as positive growth control. Whereas the well with nutrient broth and *Allium sativum* extract were considered as the negative growth control.

### **Determination of Minimum bactericidal concentration (MBC)**

The minimum bactericidal concentration (MBC) assay was performed as an appendage to the MIC test to determine the lowest concentration of plant extract that is lethal to the target bacteria in vitro. From each well without evident growth in the MIC test, a drop of the broth was streaked across the surface of the nutrient agar plate using the inoculation loop. The dilution of the sub-cultured MIC well was recorded on each plate and the plate were then incubated for 24 hours at 37°C. After incubation, the MBC plates was observed for colony growth or any lack of growth for each dilution that was sub-cultured. If there were no visible growth, the plant extract will be considered to be bactericidal at that particular dilution. On the contrary, if there is any growth, the plant extract will be deemed bacteriostatic but not bactericidal at that particular dilution.

### **Statistical analysis**

The data were expressed as mean  $\pm$  standard deviation.

## **3. RESULTS**

The zones of inhibition from different concentration of garlic extracts against *E. coli* and *S. aureus* are presented in figures 1 and 2, respectively. The activity was portrayed by the zones of inhibition for each pathogen that is shown in Figures 3 and 4. All different concentrations of garlic extract displayed different zone of inhibition indicated that there was antibacterial activity.

All garlic extracts concentration exhibited varying degrees of antimicrobial effects against the tested microbes. The high concentration of garlic extracts (D) yielded highest inhibition against *S. aureus* (33 mm); the least zone of inhibition was observed in the low concentration of garlic extracts against *E. coli* (19 mm).

Regardless of concentration of garlic extracts used in this study, the zone of inhibition was larger when compared with those produced by the antibiotic Amikacin and Vancomycin. The zone of inhibition of garlic extracts at 100% concentration was nearly double than the antibiotic Amikacin with regard to *E. coli* (31 vs 16 mm), and *S. aureus* (33 vs 13 mm) respectively.

The MIC of the 100% garlic extract was 6.25 mg/ml and 1.56 mg/ml for *E. coli* and *S. aureus* respectively as shown in Figure 4 and 5. The MBC was confirmed by absence of bacterial growth of the tested bacteria inoculated from inhibition well starting from the highest concentration (100 mg/ml) until the concentration corresponding to the lowest MIC. Garlic extract showed absence of bacterial growth for all inculcation which indicates potentially bactericidal activity against *E. coli* with MBC of 6.25 mg/ml as shown in Figure 7. On the other hand, garlic extract showed MBC against *S. aureus* at 100 mg/ml as shown in Figure 8.

## **4. DISCUSSION**

Antimicrobial resistance has been increasing from time to time. This led the scientist to focus on discovering the antimicrobial activity of different plants and herbs. One of such botanicals is plants is garlic (*Allium sativum*). Garlic has been used for long time by different societies as traditional medicine against infectious diseases. It is available in the form of supplements capsules and powders. Long-time back, Louis Pasteur described the antimicrobial effect of garlic and onion juices against both Gram-positive and Gram-negative bacteria (Khashan 2014). In this article, garlic extract with various concentrations ranged from 25 - 100%

showed antibacterial activity against both *E. coli* and *S. aureus* which represent both Gram-positive and Gram-negative bacteria. Ankri and Mirelman (1999) showed that the antibacterial effect of garlic is mainly due to allicin. Thus, when the garlic extract is highly concentrated, it is capable to inhibit larger zone of bacteria (Ankri and Mirelman 1999, Yadav, Trivedi *et al.* 2015). Based on the results showed in Figure 1 and Figure 2, the highest zone of inhibition was showed at 100% concentration of garlic extract for both organisms *S. aureus* and *E. coli*. Safithri *et al.* (2012) reported that the higher concentration of garlic water extract showed a wider inhibition zone against *S. agalactie*, *S. aureus*, and *E. coli* which support our results (Safithri, Bintang *et al.* 2012). Another previous study showed that the antimicrobial activity of allicin is mainly by immediate and total inhibition of RNA synthesis and partially inhibition of DNA and protein syntheses (Feldberg, Chang *et al.* 1988). This suggests that RNA is the primary target of allicin action. The structural variations of different bacterial species could also contribute to the difference in the susceptibility to garlic components. Furthermore, other study showed antimicrobial activity for the garlic against fungi (Davis, Perrie *et al.* 2003). Based on the results showed in the Figure 5, the MIC of garlic extract on *E. coli* with the concentration of 6.25 mg/ml whereas in Figure 6, the MIC of garlic extract on *S. aureus* occurred with the concentration of 1.56 mg/ml. These result slightly different with reported results that showed MIC of garlic extract is 7.5 mg/ml to prevent *S. aureus* growth (Mozaffari Nejad, Shabani *et al.* 2014). These results concluded that garlic has antibacterial properties against other Gram-positive and Gram-negative bacteria, which should be investigated in further studies.

The MBC of the garlic extract on *E. coli* occurred at the same concentration as MIC. There was no visible growth of bacteria from the highest concentration until the MIC concentration. Thus, we can conclude that the garlic extract with the concentration of 6.25 mg/ml is being not only able to inhibit the *E. coli* growth, but it could also kill the bacteria at that concentration. On the other hand, garlic extract showed MBC against *S. aureus* at 100 mg/ml as there is a bacterial growth from lowest concentration 1.56 mg/ml (MIC) until the concentration of 50 mg/ml suggesting that the effect is only bacteriostatic. The bactericidal effect is clear at concentration of 100 mg/ml as shown in figure 8. The results of MIC and MBC of the garlic extract suggested that it can be used as antibacterial agent. Bacterial species included in this study were chosen for their medically importance ad to represent Gram-positive and Gram-negative bacteria.

## 5. CONCLUSION

This screening study showed that garlic have potentials as antibacterial agents for both Gram-positive and Gram-negative bacteria. Additional studies are necessary to assess the possibility of using garlic as an antibacterial drug separately or in combination with conventional antibacterial drugs to evaluate the synergetic effect that can help in treatment infectious diseases.

## 6. ACKNOWLEDGEMENTS

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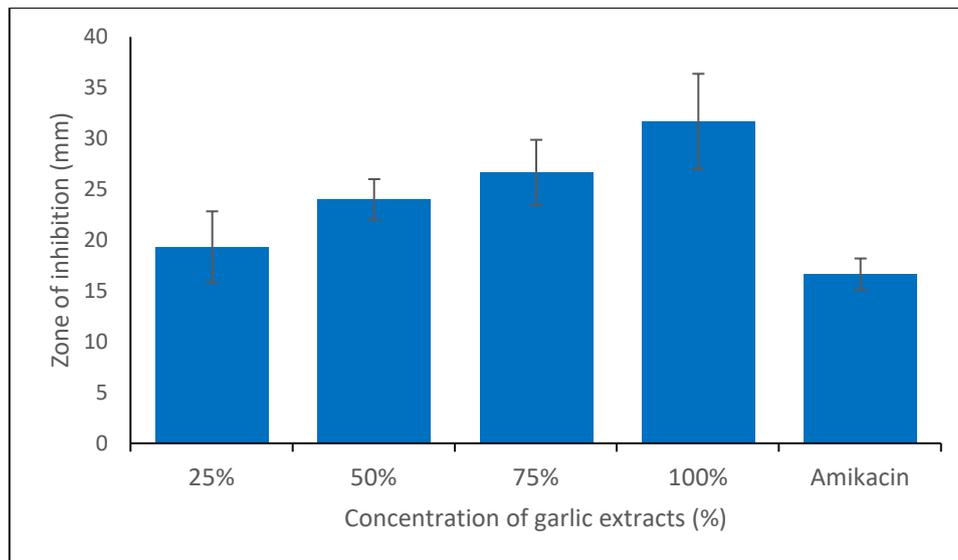


Figure 1. Mean (SD) of antibacterial activity of garlic extracts at 25%, 50%, 75% and 100% concentration against *E. coli*. Amikacin (10 µg/disk) was used as a standard disk. Bacteria were spread on Mueller–Hinton agar plates at a McFarland standard of 0.5 and incubated for 24 hours at 37°C.

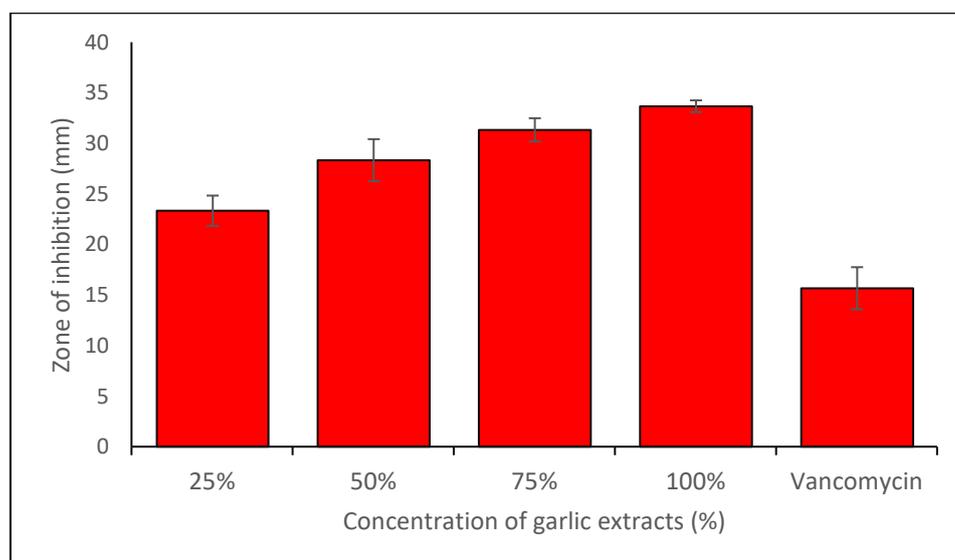


Figure 2. Mean (SD) of antibacterial activity of garlic extracts at 25%, 50%, 75% and 100% concentration against *S. aureus*. Vancomycin (10 µg/disk) was used as a standard disk. Bacteria were spread on Mueller–Hinton agar plates at a McFarland standard of 0.5 and incubated for 24 hours at 37°C.



Figure 3. The picture shows the bacteria cultured agar plate with zone of inhibition of garlic extract against the *E. coli*: A) Positive (Amikacin disc) and negative control (sterile plain disc impregnated with 10 $\mu$ l of sterile distilled water). B) Zone of inhibition of 4 different concentrations of garlic, respectively, 25% (A), 50% (B), 75% (C) and 100% (D).

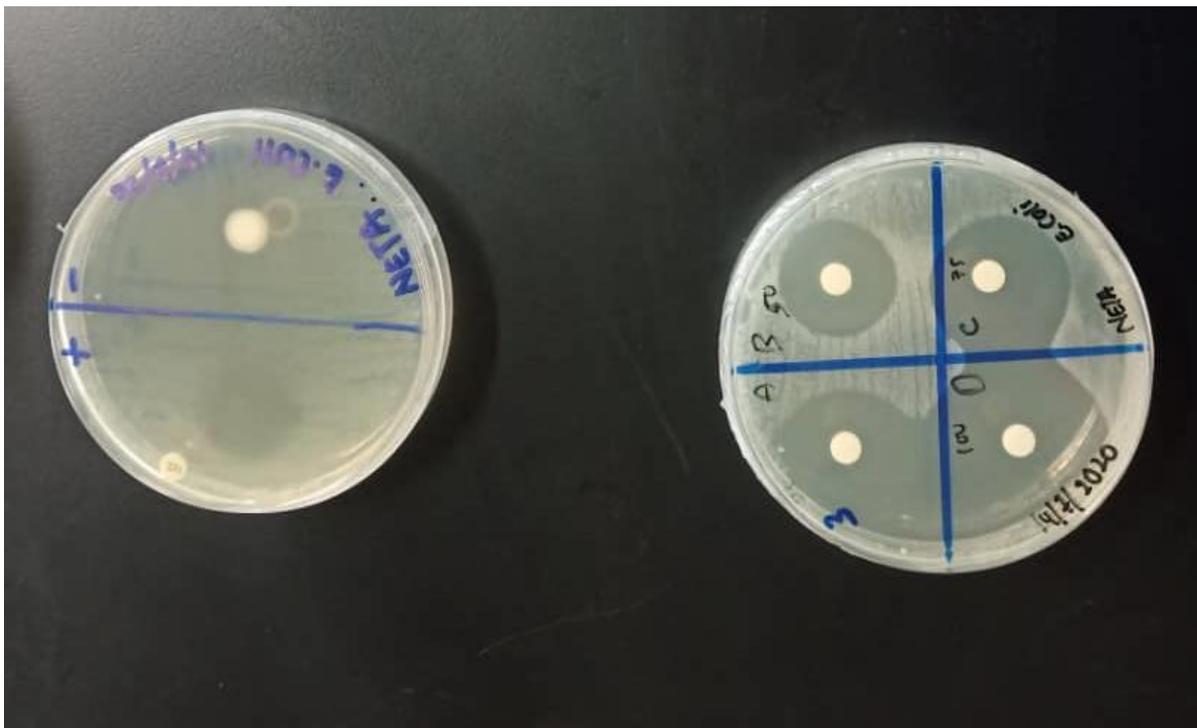


Figure 4. The picture shows the bacteria cultured agar plate with zone of inhibition of garlic extract against the *S. aureus*: A) Positive (Vancomycin disc) and negative control (sterile plain disc impregnated with 10 $\mu$ l of sterile distilled water). B) Zone of inhibition of 4 different concentrations of garlic, respectively, 25% (A), 50% (B), 75% (C) and 100% (D).

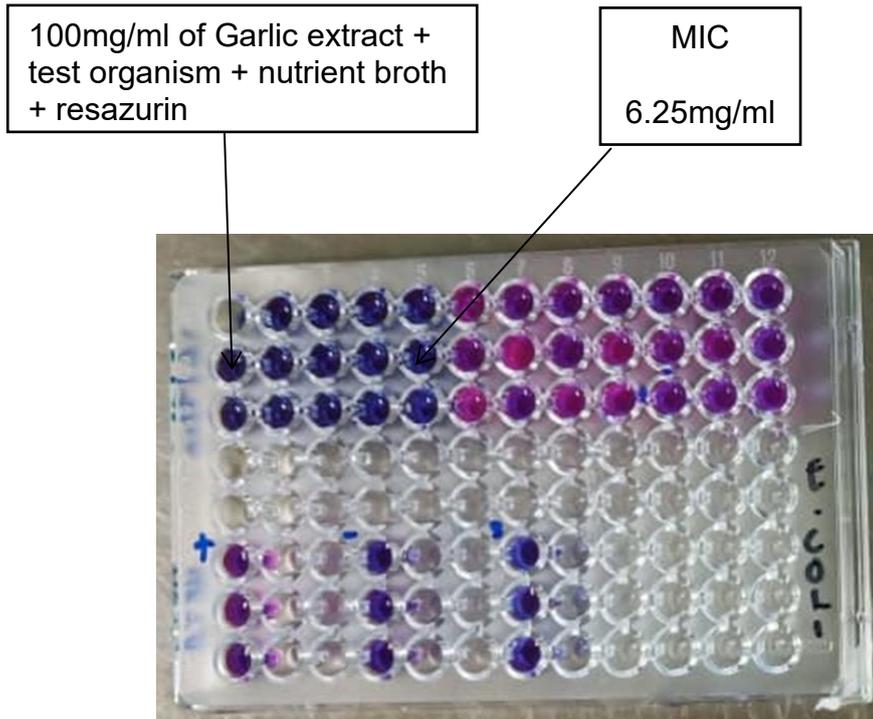


Figure 5 shows the result of MIC for 100% concentration of garlic extract on *E. coli*.

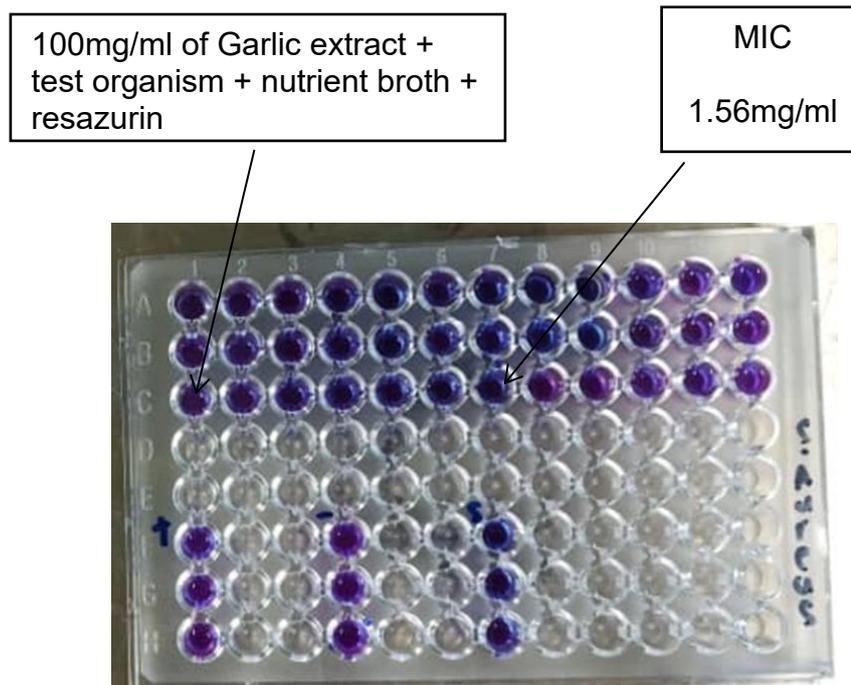


Figure 6 shows the result of MIC for 100% concentration of garlic extract on *S. aureus*.

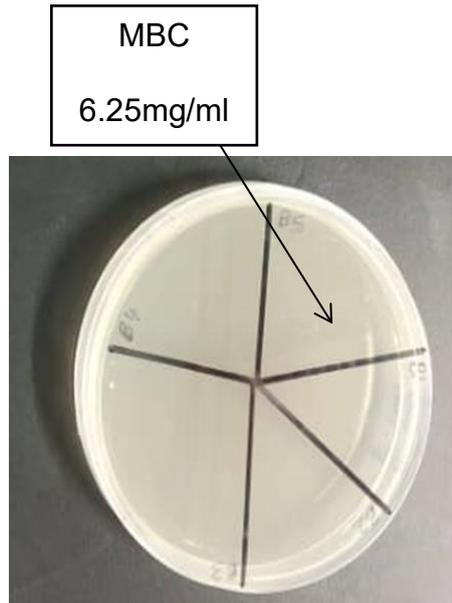


Figure 7 shows the result of MBC of garlic extract on *E. coli*.

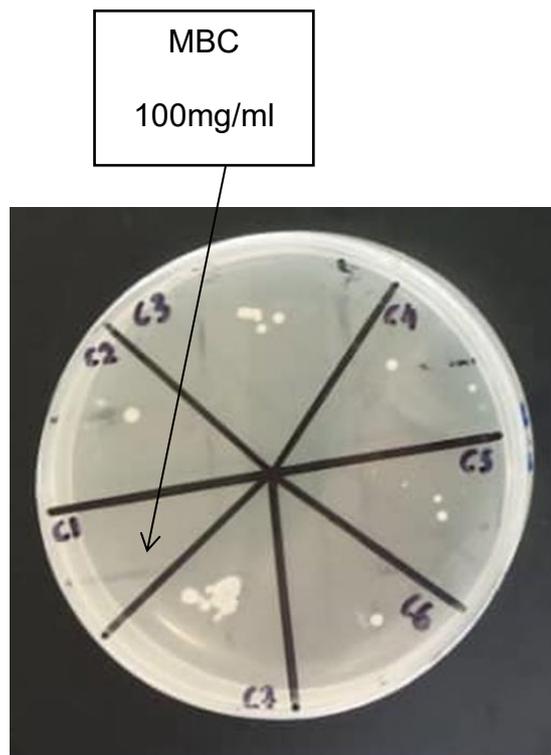


Figure 8 shows the result of MBC of garlic extract on *S. aureus*.