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# Garlic As Antibacterial in Lactose Fermenter Enterobacteriaceae

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## ABSTRACT

*This study attempts to investigate the antibacterial effectiveness of three varieties of garlic (single garlic, kating garlic, and black garlic) on the growth of Enterobacteriaceae bacteria (Escherichia coli, Enterobacter* sp, *Klebsiella* sp. *from chicken meat samples. Treatment Kating garlic, single garlic, and black garlic at concentrations of 20%, 40%, 60%, 80%, 90%, 100%. This study observed the inhibition zone on Muller Hinton Agar (MHA) media that had been planted with bacterial isolates. The inhibition test uses the disk diffusion method. The findings revealed that kating garlic and single garlic showed inhibition zones against Escherichia coli, Enterobacter* sp., *and Klebsiella* sp. *bacteria. Meanwhile, black garlic did not show an inhibition zone against E. coli bacteria, but showed an inhibition zone against Klebsiella* sp., *Enterobacter* sp., *and Kating garlic had the largest bacterial inhibition zone against E. coli, Klebsiella* sp., *Enterobacter* sp., *followed by single garlic.*

***Keywords** : black garlic; enterobacteriaceae; garlic; single garlic*

## INTRODUCTION

Garlic is a food ingredient often used and easily available in various countries such as North America, China, India, Southern Europe, Asia, and parts of northern Nigeria (Tijjani *et al.*, 2017). This plant species is known to have higher antimicrobial activity compared to various Allium plants because it comprises a number of hydrophobic antimicrobial compounds, such as vinyldithiins, allicin, diallyl polysulfide, and ajoenes (Mahros *et al*., 2021). Fresh garlic produces a very special smell when crushed, the special smell is mainly caused by the compound allicin disulfide which has been widely reported to have activity as an antibiotic. The results obtained from the study show that Gram negative (*E. coli*) and Gram positive (*Staphylococcus aureus*) can be against by the anti-bacterial effect from the garlic (Strika *et al.*, 2017).

Garlic types and products are starting to be marketed, starting from single garlic, processed garlic such as black garlic, smoked garlic, garlic oil, macerated garlic oil. Single garlic and black garlic are widely used in the treatment of a disease. Black garlic is created by rapidly heating whole garlic bulbs at high temperature (70°C) and high humidity (90%). The flavour of black garlic is sweeter making it easier to consume,

compared to fresh garlic, black garlic exhibits stronger antioxidant and free radical abilities. Black garlic also has antimicrobial activity against several common pathogens such as *methicillin-resistant Staphylococcus aureus* (Che Mohd Ramli *et al*., 2020).

Garlic is an excellent alternative as an antibacterial used today (Magryś *et al.*, 2021), therefore the researchers wanted to know the comparison of the antibacterial activity of several types of garlic (onion, single onion and black garlic against Enterobacteriaceae from field samples taken from chicken meat. Tests on bacterial isolates from the Enterobacteriaceae group on the basis that this bacterium is a pathogen in humans (Sorbara *et al.*, 2019).

The Enterobacteriaceae bacteria group includes many bacteria normally clinical cultures isolated, including *Enterobacter spp, Klebsiella spp.,* and *E. coli* (Tilahun *et al.,* 2021). These bacteria are widely distributed in the healthy human and animals intestines and have also been isolated from environmental samples as well as livestock, especially broiler chickens *(*Projahn *et al.,* 2018). *E.coli* is an indicator of faecal contamination in food and the amount is usually more correlated with the number of Enterobacteriaceae (Belluco *et al.*, 2016).

## MATERIALS AND METHODS

The bacterial isolates used were taken from field samples of chicken meat. The bacteria used are enterobacteriaceae which ferment lactose (bacterial colonies with red color on MacConkey Agar media), including *E. coli, Klebsiella* sp., *Enterobacter* sp. These three bacteria have been proven through examination of colony morphology, microscopic examination and confirmed by biochemical tests TSIA, SIM, SCA, Urease, MR and VP.

The garlic used included single garlic obtained from Kefamenanu NTT, kating garlic obtained from the Dukuh Kupang Surabaya market and black garlic purchased from an E-commerce. Garlic was prepared by chopping it using a sterile blade and then diluting it with 10% DMSO with concentrations including 20%, 40%, 60%, 80%, 90% and 100%. How to make 10% garlic for each type by weighing 0.1 gram added with 1 ml of DMSO. A 100% concentration is made by dissolving 1 gram of garlic in 10% DMSO solvent

Testing the antibacterial activity of kating garlic, single garlic and black garlic using the disk diffusion technique. Mueller Hinton Agar from Himedia with number of catalog M173 was filled with 0.1 ml of the bacterial suspension which had been standardized with MacFarland 0.5. The bacterial inoculum was spread using a cotton swab. Each type of garlic at various concentrations

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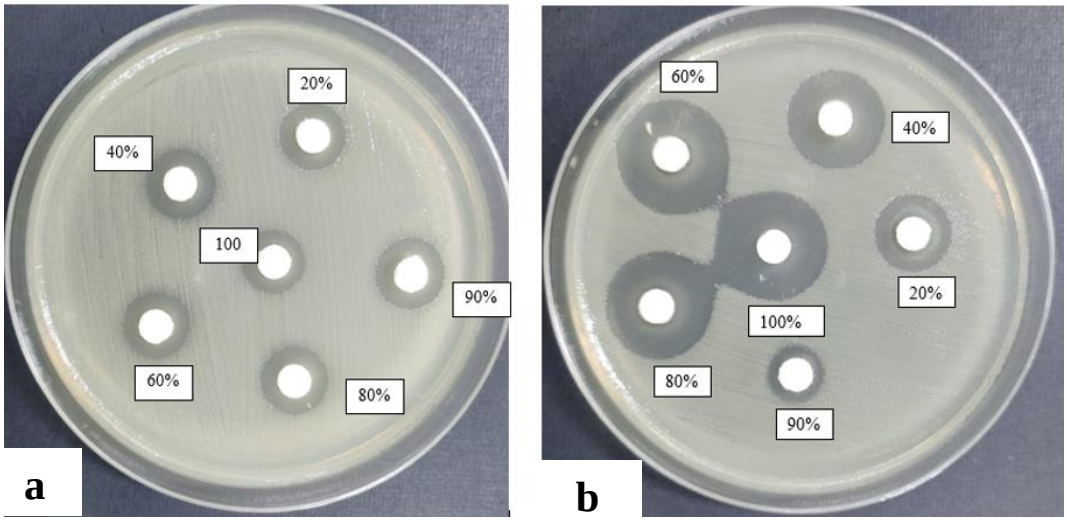
was prepared, the paper disks were soaked in the garlic suspension for 15 minutes then drained and after that they were affixed to Mueller Hinton agar media which had been flush with the bacterial suspension. Inoculated petri dishes were incubated for 24 hours at 37 ℃ and the findings were obtained by measuring the inhibition zone of the antibiotic. The research results are presented descriptively.

## RESULT AND DISCUSSION

The results showed that kating garlic and single onion could inhibit Enterobacteriaceae (*E. coli*, *Enterobacter* sp. and *Klebsiella* sp). The highest inhibition zone was formed in Enterobacter isolates, in kating garlic with a concentration of 60% and single onion with a concentration of 100%. This type of garlic is able to inhibit better than single onions.




Picture 1. (a) The inhibitory zone of garlic cloves against *E. coli* (b) The inhibitory zone of single garlic against *E. coli*.

Garlic contains allicin, a chemical compound with antibacterial characteristics that acts as a natural antibiotic. The allicin compound has the ability to damage bacterial cell walls, to interfere with the process of RNA synthesis. The way the allicin compound damages the bacterial cell wall is by inhibiting the production of dipeptidoglycan, which plays a role in providing strength and rigidity to the bacterial cell wall. On the other hand, allicin compounds inhibit RNA synthesis by creating very strong interactions with bacterial enzymes, especially DNA which is the mechanism by which allicin compounds suppress the synthesis of DNA dependent RNA polymerase, resulting in inhibition of RNA making in bacteria (Mohammed *et al.,* 2021)

According to research (Muhammad *et al.*, 2014) Flavonoids have antibacterial properties by forcing denatured bacterial proteins. Flavonoids are phenolic chemical derivatives that have the ability to bind to bacterial cells through a mechanism called adsorption. Hydrogen bonds are created as part of the interactions during this process. Phenol can promote the development of protein complexes with brittle bonds at low concentrations. When phenol penetrates cells, it undergoes rapid breakdown, causing protein deposition and denaturation. Phenol has the ability to inhibit the function of bacterial enzymes, interfere with bacterial metabolism and make it difficult for bacteria to survive.

Table 1. Inhibition zone results of kating garlic, black garlic and single garlic against *E. coli*

| Concentration | Kating garlic (mm) | Black garlic (mm) | Single garlic (mm) |
|---|---|---|---|
| 20% | 23 | 0 | 11 |
| 40% | 23 | 0 | 17 |
| 60% | 25 | 0 | 19 |
| 80% | 21 | 0 | 21 |
| 90% | 21 | 0 | 21 |
| 100% | 21 | 0 | 21 |

Black garlic is able to inhibit Klebsiella and Enterobacter bacteria. The larger the concentration (100%) the greater the resulting inhibition zone. However, black garlic is not able to inhibit *E. coli* bacteria (no inhibition zone is formed). The findings of this study are in accordance with study conducted by Abiy and Berhe, (2016) the study found that higher concentrations produced larger clear zones, while lower concentrations produced smaller clear zones.

Table 2. Inhibition zone results of kating garlic, black garlic and single garlic against *Enterobacter* sp.

| Concentration | Kating garlic (mm) | Black garlic (mm) | Single garlic (mm) |
|---|---|---|---|
| 20% | 24 | 7 | 11 |
| 40% | 24 | 7 | 16 |
| 60% | 25 | 7 | 19 |
| 80% | 22 | 7 | 23 |
| 90% | 22 | 8 | 24 |
| 100% | 22 | 8 | 25 |

The results showed that black garlic did not show an inhibition zone against *E. coli*. Black garlic is a heat-treated processed product and then fermented from whole garlic bulbs. When garlic is heated,

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physicochemical changes occur, changes in colour, texture, and taste, as well as modification of the nutritional content of garlic (Bae *et al*., 2014). Phytochemicals from flavonoid compounds from garlic were higher than black garlic. *(Choi et al.*, 2014).

There are many factors affect the size of the inhibition zone formed(Harun *et al.,* 2021). In the process of making black garlic, several factors such as fermentation time, temperature and type of solvent (Bar *et al*., 2022) can produce different inhibition zones. In the experiment, water produced the highest yield, with ethanol, chloroform and petroleum ether following in descending order *E. coli* is very vulnerable compared to *S. aureus*.

Table 3. Inhibition zone results of kating garlic, black garlic and single garlic against *Klebsiella* sp.

| Concentration | Kating garlic (mm) | Black garlic (mm) | Single garlic (mm) |
|---|---|---|---|
| 20% | 13 | 8 | 9 |
| 40% | 12 | 8 | 12 |
| 60% | 12 | 8 | 12 |
| 80% | 12 | 9 | 12 |
| 90% | 12 | 9 | 12 |
| 100% | 12 | 9 | 12 |

In addition, the method of processing and storage at freezing temperatures also affects the size of the inhibition zone. Fresh garlic cloves have no allicin. However, after being crushed and chopped with the enzyme alliinase, it is released.

Antimicrobial activity of frozen garlic against 9 bacteria, while Frozen garlic has antimicrobial activity against 9 bacteria, while sliced Turkish garlic has antimicrobial effect against 6 bacteria (Yetgin *et al*., 2018).

## CONCLUSION

According to the findings of this study, kating garlic exhibits the best antimicrobial activity indicated by the largest inhibition zone compared to single garlic and black garlic

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