

Antibacterial Potency of Black Garlic Extract from *Allium sativum* on *Escherichia coli*

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ABSTRACT

Background: Diarrhea is still one of the global issues especially in developing countries. Diarrhea can be caused by either an infectious agent or a non-infectious agent. *Escherichia coli* is one of the infectious agents that is responsible for causing diarrhea. Garlic (*Allium sativum*) is a plant that has a good antibacterial activity potential because of its organosulfur and phenolic compound. Black garlic is the product of spontaneous fermentation of garlic that has improved its bioactive compounds.

Content: Antibacterial potency of black garlic extract on *Escherichia coli* is shown on the resistance zone that formed where the lowest zone diameter is 9,67 mm while the highest zone is 24 mm. The fermentation of black garlic happened at the temperature of 70-80°C with 75-80% for 28-40 days.

Conclusion: Black garlic is shown to exhibit antibacterial activity against bacteria such as *Escherichia coli*. This fermented product has the potential to be a therapeutic agent for diseases caused by *Escherichia coli*.

Keywords: Garlic; Antibacterial; *Escherichia coli*



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Introduction

Diarrhea is still one of the major global issues with high morbidity and mortality rate in some countries, especially in developing ones (Hartati et al, 2018). According to data from World Health Organization (WHO) in 2013, every year there are approximately 1,7 billion cases of diarrhea with 760.000 mortality numbers on children under the age of 5. ⁽¹⁾

In Indonesia, the incidence of diarrhea for all ages according to Rapid Survey in 2019 is estimated at 270/1.000 morbidity rate and 843/1.000 for children. The total finding of diarrhea in medical facilities is 7.265.013 and 3.979.790 of them are children (Kemenkes RI, 2020). ⁽²⁾

Based on the profile of Sulawesi Selatan's public health office per 2017, there is 257.108 citizen with diarrhea where 44.914 cases are from Makassar (Dinkes Sulsel, 2018). That data shows that diarrhea is still a common problem in current society. ⁽³⁾

Diarrhea can be caused by an infectious agent and a non-infectious agent. The most common non-infectious agent is food allergies. Infectious agents, on the other hand, are caused by bacteria, viruses, or parasites. *Escherichia coli* (*E. coli*) is the most common pathogen group that causes diarrhea. Two among them are ETEC (*Enterotoxigenic Escherichia coli*) and EHEC (*Enterohemorrhagic Escherichia coli*). ETEC is the most common cause of diarrhea in children in developing country while EHEC causes diarrhea from contaminated meat (Leon T, 2020). ⁽⁴⁾

Garlic (*Allium sativum*) is one of the plants that have many medicinal properties, among them are antimicrobial. Its contents such as Allicin are useful to inhibit gram-positive and gram-negative bacteria by inhibiting the production of RNA and lipid synthesis, so the bacteria cannot thrive. The antibacterial capacity has more potential in gram-positive bacteria compared to gram-negative bacteria (Mouliya MN et al, 2018). ⁽⁵⁾

Garlic (*Allium sativum*) is also rich in chemicals that are useful as antioxidants. Organosulfur compounds such as allicin, adenosine, ajoene, flavonoid, and saponin provide many benefits, especially in the health sector (Wibisono Y et al, 2020). ⁽⁶⁾

The results of the study prove that garlic extract (*Allium sativum*) has antibacterial potential against gram-positive bacteria (*Staphylococcus epidermidis*, *Streptococcus viridans*) and gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*). However, the extract has a greater inhibitory effect on gram-positive than gram-negative bacteria (Airaodion AI et al, 2020) ⁽⁷⁾

Black Garlic is the result of fermenting Garlic (*Allium sativum*) over a certain period with a high temperature (60-90°C) and a high humidity (80-90%) environment. Its physicochemical changes increase the bioactivity of its components. Garlic's rich allicin content is converted into antioxidant components such as alkaloids and flavonoids. Allicin which gives a lot of flavor to garlic is broken down into other components such as diallyl sulfide, diallyl disulfide, and ajoene (Kimura S et al, 2017). ⁽⁸⁾

The description above encourages the researcher to find out more about the potential of black garlic in increasing the effectiveness of garlic as an antibacterial product, especially on gram negative-bacteria. The results of this study are expected to prove the effect of black garlic extract as a more efficient antimicrobial to inhibit the work of *Escherichia coli* bacteria.

Subheading

This research uses the literature review method with a narrative review design. Journals that are taken as data are from both international and national journals released from the year 2017-2021 that are considered relevant to the topics.

The inclusion criteria are International Journal, National Journal, or textbook with keywords consisting: Antibacterial activity of black garlic on *Escherichia coli*, Disc diffusion method of black garlic on *Escherichia coli*, Black garlic effect on *Escherichia coli*, from the year 2017-2021. Electronic-based media includes sources from Elsevier / Clinical Key, PubMed and, Google Scholar.

While the exclusion criteria are journals that are found not in the form of full text (can't be fully accessed) and journals that are not from the web with international or national standards.

From keyword searches, obtained results as many as 203 journals. All journals are included in the search and then filtered. In the end, 8 remaining journals matched the inclusion criteria, which will then be used for this literature review.

Of the 8 journals, 6 journals were research conducted using a true experimental design method to see the antibacterial effect of black garlic on *Escherichia coli*. In the research of Thalia et al (2020) the largest inhibition zone was 12.8 mm with black garlic fermented at 80°C for 40 days.⁽⁹⁾ From research done by Setiyoningrum et al (2021) obtained the largest inhibition zone of 24 mm with ethyl acetate solvent and 18.9 mm with isopropanol solvent using black garlic which was fermented at a temperature of 72°C for 4 weeks.⁽¹⁰⁾ In the research of Chang et al (2021) the largest inhibition zone was 9,67 mm with Michiu Tou wine as a solvent on black garlic that was fermented at 70°C for 35 days.⁽¹¹⁾ Astuti et al (2018) used black garlic's essential oil with the largest inhibition zone at 13,16 mm.⁽¹²⁾ Kang et al (2017) got 11,65 mm for its largest inhibition zone using black garlic that sold in the market.⁽¹³⁾ Sasaki et al (2017) also proved that black garlic fermented at 70°C for 30 days gives an antibacterial effect on *Escherichia coli*.⁽¹⁴⁾

While the other 2 journals use the literature review method. In the research of Botas et al (2019), it was found that the antibacterial test of black garlic showed sensitive results to *Escherichia coli* 0157:H7.⁽¹⁵⁾ In Ahmed et al (2021) research it was found that black garlic, after going through pre-clinical and clinical studies, showed clear evidence that its consumption can be a supportive therapy for various diseases in humans.⁽¹⁶⁾

Based on 8 journals that have been researched, it was found that in general black garlic extract has an antibacterial effect against *Escherichia coli*. The fermentation process carried out by each study to obtain black

garlic extract also varied. Of the 8 journals, 4 journals did not explain the fermentation of black garlic samples, while in the other 4 journals, various black garlic fermentation processes were found. In a study conducted by Thalia et al (2020) the most effective fermentation is at the temperature of 80°C with an oven time of 40 days. Research by Setiyoningrum et al (2021) said that 4-week fermentation with the temperature of 72°C and 80% humidity showed the best antibacterial activity. In a journal by Chang et al (2021) it was stated that the most optimal black garlic fermentation is for 35 days at a temperature of 70°C with 85% humidity. Meanwhile, in the study of Sasaki et al (2017) black garlic extract was fermented at a temperature of 70°C with 75% humidity for 30 days.

Based on those 4 studies, it can be seen that the average fermentation is done at the temperature of 70-80°C with 75-85% humidity for 28-40 days. This is following the journal *Novel Nutritive Garlic Product “Black Garlic” : A Critical Review of Its Composition, Production, and Bioactivity* by Alihanoğlu S et al (2017) who said that fermentation to produce the best characteristic black garlic is done at high temperatures (70-80°C) with 70-90% humidity for about 21 days.⁽¹⁷⁾

In a study conducted by Chang et al (2021) it was said that fermented garlic had more bacteriosatic capacity due to the presence of antibacterial agents besides allicin in black garlic. This is following the journal *Converting organosulfur compounds to inorganic polysulfides against resistant bacterial infections* by Xu Z et al (2018) which stated that the bacteriostatic capacity of garlic was mostly obtained from Allicin derivatives, such as diallyl trisulfide (DATS), diallyl disulfide (DADS), diallyl sulfide (DAS).⁽¹⁸⁾ And in accordance to journal *Biological Activities of Black Garlic Fermented with Lactobacillus Plantarum PN05 and Some Kinds of Black Garlic Presenting Inside Vietnam* by Ngan N et al (2017) this component increased by five to six times in the form of a more stable organosulfur compound namely SAC.⁽¹⁹⁾

The antibacterial power of black garlic extract itself is obtained from the constituent components of black garlic which are rich in organosulfur and phenolic compounds. The organosulfur component that increased dramatically due to the fermentation process is a compound named S-allylcysteine (SAC), which plays a major role in antioxidant activity to ward off free radicals. The content of phenolic bioactives such as polyphenols and flavonoids also increases due to the heating effect during fermentation. Antimicrobial activity in black garlic is not only obtained from the allicin content which is still present in black garlic but can also come from the content of phenolic compounds by inhibiting RNA synthesis quickly and thoroughly and partially DNA and protein synthesis. This damages the cell membrane resulting in inhibition of the activity and biosynthesis of specific enzymes needed in bacterial metabolic reactions

In the journal *Influence of thermal processing on the bioactive, antioxidant, and physicochemical properties of conventional and organic agriculture black garlic (Allium sativum L.)* by Najman k et al (2020) it is known that *S-allylcysteine* (SAC) is the most important bioactive compound that plays a major role in

black garlic's pharmacological effects.⁽²⁰⁾ *S-allylcysteine* (SAC) is a component that exhibits very high antioxidant activity. This is following the research of Thalia et al (2020) which showed that the antioxidant activity and total phenolic compound of black garlic were twice those of unfermented garlic.

In the research of Botas J et al (2019) it was found that black garlic had antibacterial activity on all bacterial isolates it was tested on. The study showed that black garlic produced bactericidal activity although the amount was not significant. Minimal bactericidal concentration (MBC) was found to be most visible in *Escherichia coli* and *Staphylococcus aureus*. This incident can be related to the flavonoid content in black garlic. In the journal *Efektivitas Daya Hambat Bakteri Ekstrak Bawang Dayak Terstandarisasi Flavonoid Terhadap Enterococcus Faecalis (In Vitro)* by Armanda F et al (2017) it is said that flavonoids are phenolic compounds that have a toxic effect on bacteria due to their ability to damage the hydrogen bridge bonds of double-stranded DNA strands. Flavonoid compounds will contact the DNA in the cell nucleus and through the difference in polarity between the lipids that make up DNA and the alcohol groups on the flavonoid compounds a reaction will occur, thereby damaging the lipid structure of the DNA and eventually the bacterial cell nucleus will also lyse and die.⁽²¹⁾

Research conducted by Ahmed T (2021) showed the results of giving black garlic in-vivo to various types of rats. These studies show a positive therapeutic effect against various diseases. This is following with the journal *Black Garlic and Its Therapeutic Benefits. In: Medicinal Plants - Use in Prevention and Treatment of Diseases* by Tran G (2020) says that consumption of black garlic can be a supportive therapy for diseases because of its anticancer, antiobesity, antioxidant, and anti-inflammatory effects.⁽²²⁾

Conclusion

From the results and discussions, it can be concluded that black garlic extract has an antibacterial effect against *Escherichia coli*. The inhibition zone formed after giving black garlic extract averaged at 9,67 mm – 24 mm. The average black garlic fermentation is carried out at a temperature of 70-80°C and 75-80% humidity for 28-40 days. The greatest bacterial inhibition was found in 4 weeks of fermentation with a temperature of 72°C and 80% humidity with *Ethyl acetate* dan *Isopropanol* solvents. Black garlic fermentation increases the antioxidant activity and total phenolic compounds of garlic (*Allium sativum* L). Organosulfur compounds such as S-allylcysteine (SAC) and flavonoid compounds are the active substances in black garlic that play the most role in inhibiting *Escherichia coli*.

Suggestion for further research, research should be carried out to study the different use of different solvents while making black garlic about the antibacterial effects of said black garlic on *Escherichia coli*. In addition, it is necessary to study the effect of black garlic extract on other bacteria. The purpose is to see the spectrum of antibacterial effects of black garlic.

Conflict of Interest

No potential conflict of interest relevant to this article was reported

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