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Submission date: 30-Dec-2021 12:19PM (UTC+0700)
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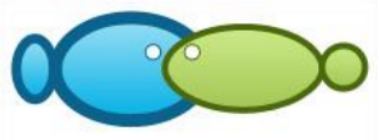
File name: Turn_Saiman_et_al.doc (624K)
Word count: 3562
Character count: 20269



Potential of ethanolic garlic *Allium sativum* L. as an antimicrobial against *Escherichia coli* bacteria and *Candida albicans* fungus

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Abstract Garlic *Allium sativum* L. has biological and pharmacological effects because of its volatile oil content. This study aims to examine the potential of the ethanolic extract of garlic as an antimicrobial against *Escherichia coli* bacteria and the *Candida albicans* fungus. This study was conducted at the Food Technology Laboratory, Faculty of Agriculture, Bosowa University, Makassar with the method used for extraction was an experimental method using ethanol as a solvent, antimicrobial testing using the agar diffusion method with pure bacterial colonies of *E. coli* ATCC 25922 and pure cultures of *C. albicans* ATCC 10231, positive control for bacteria using chloramphenicol, for fungi using ketoconazole, and negative control using distilled water. The concentration of garlic extract used was 10%, 20% and 30%. The results obtained were garlic extract for *E. coli* bacteria with a concentration of 20% was bacteriostatic with an inhibition zone formed at 24 hours of 15.50 mm, at 48 hours reduced to 15.20 mm. Concentration of 30% with the inhibition zone formed at 24 hours of 16.90 mm and at 48 hours being 16.60 mm also bacteriostatic. For *C. albicans* at a concentration of 10% it is fungistatic with an inhibition zone at 48 hours of 10.80 mm and at 72 hours it is reduced to 10. 40 mm, while at concentrations of 20% and 30% it is fungicidal, with an inhibition zone at 48 hours. Garlic *A. sativum* L. has the potential to be developed as an antimicrobial against *E. coli* and the *Candida albicans*.
Key Words: garlic *Allium sativum,* antimicroba, *Escherichia Coli, C. albicans*

Introduction. Pathogenic microbes are microorganisms that infect humans the most and cause various diseases. *Echerichia coli* is a gram-negative bacterium that can cause disease in humans and animals by producing enterotoxins (Pakbin Babak, et al., 2021). *E.coli* bacteria are normally found in the large or small intestinal tract of healthy children and adults with numbers up to 109 CFU/g (Markowiak Paulina and Katarzyna liżewska., 2017). These bacteria are known as indicators of faecal contamination. Under low hygienic conditions, enterotoxigenic *E. coli* infection can cause watery diarrhea with abdominal cramps, fever, malaise and vomiting (Park Sang-Hun, et al., 2018). Likewise, candidiasis is a disease caused by the Candida. Candidiasis can be acute or sub-acute which usually infects the mouth, vagina, nails, and lungs (F. Célia Rodrigues, et al., 2019). More than 150 species of Candida have been identified, but 70% of Candida that infects humans is caused by *Candida albicans*.

To overcome various infectious pathogenic diseases, the use of drugs is increasing. However, the continuous use of drugs causes resistance, especially to drugs that are classified as antibiotics (Suhas Tushar Khare, et al., 2021). To overcome multi-drug resistance, it is necessary to develop other alternatives by looking for natural ingredients that do not have side effects, which can be developed as basic ingredients for drugs (D. Pooja Gupta and Tannaz J. Birdi., 2017). Garlic *A. sativum* is one of the spices that plays a role in adding to the aroma and taste of food and also has properties that are important for health. Garlic has biological and pharmacological effects such as antitumor, anti-artheroclerosis, modulating blood sugar and inhibiting cancer growth (JB Divya, et al., 2017). Garlic also has antibacterial and antifungal properties due to its allicin content (Khairan Khairan, et al., 201).

Allicin can inhibit the growth of gram-positive and gram-negative bacteria by inhibiting RNA production and lipid synthesis (A.S. Beverly Reyes, Albert B. Leung., 2018). Allicin also has antifungal properties because it contains organic sulfur compounds, namely alliin which is synthesized from the amino acid cysteine (Sesha Murugan Subramanian, et al., 2020). If *A. sativum* is cut into pieces, the allinase enzyme will convert alliin into allicin, which gives rise to the distinctive aroma of garlic (Valentino Hannah, et al., 2020). Allicin which contains organosulfur compounds is responsible for antimicrobial activity, not only can penetrate cell membranes, but also organelle membranes such as mitochondria which causes organelle damage and cell death (Bagde Sushma Bhatwalkar, et al., 2021). So this research was conducted to examine the ability of garlic *A. sativum* to inhibit or kill the growth of *Echerichia coli* and *Candida albicans*. Through this study, it is hoped that garlic can be used as an alternative base material that can be developed as an antimicrobial.

Materials and Methods. The method used is an experimental method using *A. sativum* garlic which is mashed as much as 500 g then macerated using 96% ethanol solvent, then evaporated to get thick macerate. Antimicrobial bioactivity test using agar diffusion method with pure culture of *E. coli* ATCC 25922 and pure culture of *C. albicans* ATCC 10231, positive control for bacteria using chloramphenicol, while for fungi using ketoconazole, and negative control using distilled water. The concentration of garlic extract used was 10%, 20% and 30%. The solvent used was NaCl, 70% alcohol, sterile distilled water, Potato Dextrose Agar (PDA) medium (Merck), Nutrient Agar (NA) medium, ketoconazole (PT. Alpharma), and aluminum foil.

Preparation of garlic ethanol extract. Preparation of garlic extract using 96% ethanol by maceration method. The chopped garlic is air-dried until it produces a constant dry weight. A total of 500 grams of dried garlic pieces, then crushed and macerated with ethanol (1:3) for 1 Xx 24 hours, then filtered and the maserate was collected in one container. Maceration was repeated 3 times. The maceration results are evaporated to obtain thick macerate and stored in a desiccator to evaporate the remaining methanol. Then 10%, 20%, and 30% extract concentrations were made for antimicrobial testing.

Preparation of antibacterial and antifungal positive control solution. The positive control solution for bacteria was prepared from 500 mg chloramphenicol tablets by crushing one chloramphenicol tablet. After that, 50 mg was weighed and dissolved in 50 ml of distilled water, then made by taking 1 ml of the solution and adding up to 10 ml of distilled water to obtain a 5µg/50µl chloramphenicol solution. Likewise, Ketoconazole 500 mg as a positive fungal control was crushed and weighed as much as 50 mg and dissolved in 50 ml of distilled water, then 1 ml was taken and added up to 10 ml of distilled water to obtain a 5µg/50µl chloramphenicol solution.

Testing of antibacterial activity with agar diffusion method. Nutrient agar (NA) base media was poured into a petri dish and allowed to solidify, then 5 blank discs were placed. Furthermore, 10 mL of nutrient agar medium containing the suspension of the test bacteria was poured into a petri dish and allowed to solidify. The blank disc is removed so that a well is formed for the test solution (Bonnet M., et al, 2020). The test solution of garlic extract with 3 different concentrations (10%, 20%, 30%), positive control using chloramphenicol, and negative control of distilled water was dripped as much as 50 µl in different wells which was carried out in duplicate and then incubated in an incubator at 37ºC for 24 hours. The diameter of the inhibition zone formed was observed and measured using a caliper. According to Małgorzata Kikowska, et al., (2016), the inhibition area of 20 mm or more means very strong, the inhibition area is 11-20 mm (strong), 5-10 mm (moderate), and the inhibition area is 5 mm or less (weak).

Testing of antifungal activity with agar diffusion method. The antifungal activity was tested using the diffusion method using an iron cylinder buffer. Potato Dextose Agar

(PDA) media was poured into a petri dish and allowed to solidify. On the surface of the base layer 5 buffers are placed and arranged in such a way that there is a good area to observe the inhibition zone that will form. PDA medium containing the test mushroom suspension was poured into a petri dish around the blank disc. Remove the backing from the petri dish to form a well that will be used for the test solution with 3 different concentrations (10%, 20%, 30%) as well as a positive (+) and negative control (-) control solution. Repeat in duplicate in the same way. Incubated for 3x24 hours at 37°C in an incubator. The inhibition zone formed around the well was observed and the diameter of the inhibition zone was measured vertically and horizontally using a caliper. The resistance area of 20 mm or more means very strong, the inhibition area is 11-20 mm (strong), 5-10 mm (moderate), and the inhibition area is 5 mm or less (weak) (MałgorzataKikowska, et al., 2016)

Data analysis. This data was analyzed quantitatively by measuring the inhibition zone formed. The research data will be analyzed descriptively and processed in pictures.

Results.

Test of garlic extract bioactivity against E. coli

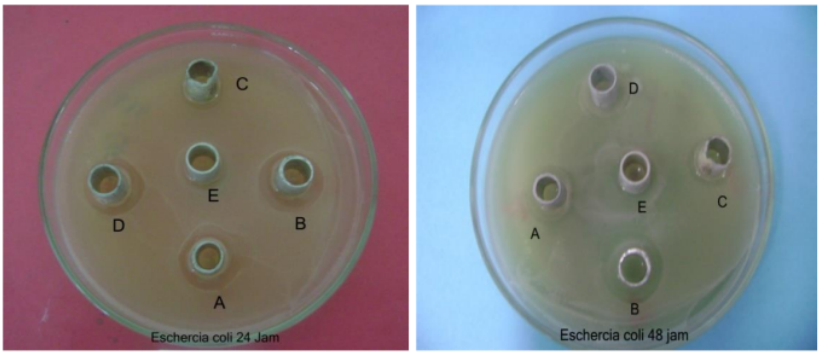

Figure 1. Garlic extract test on *E. coli* bacteria with incubation period of 24 hours and 48 hours.

Table 1

Results of measuring the bioactivity of garlic extract against *E. coli*

No	*Concentration of Compounds*	*Average of Zone Inhibition (mm)*	
		24 hours	*48 hours*
A	Garlic ethanol extract 30 ppm	16.90	16.60
B	Garlic ethanol extract 20 ppm	15.50	15.20
C	Garlic ethanol extract 10 ppm	3.15	2.70
D	Chloramphenicol (positive control)	16.90	16.50
E	Aquades (negative control)	00.00	00.00

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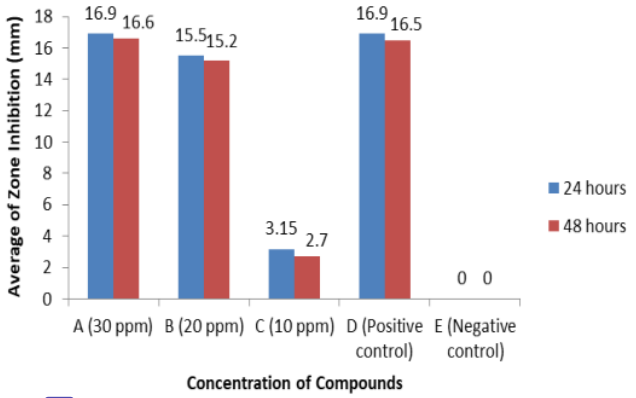

Figure 2. Histogram of inhibition diameter of garlic extract against the *E. coli*

In figure 1 and table 1, it appears that the treatment of garlic extract against *E. coli* bacteria at 24-hour incubation where the largest inhibition zone formed was at a concentration of 30% with a diameter of 16.90 mm. Then followed by a positive control of 16.90 mm chloramphenicol, then garlic extract at a concentration of 20% with an inhibition zone of 15.50 mm. For garlic extract with a concentration of 10 ppm, the smallest inhibition zone was 3.15 mm. Meanwhile, distilled water did not form an inhibition zone. At 48 hours of incubation, the largest inhibition zone at 30% concentration was 16.60 mm which showed 30% concentration was bacteristatic, while the antibiotic chloramphenicol used in the inhibition zone formed at 17.20 was bactericidal, meaning that it is deadly to *E. coli*. Meanwhile, for garlic extract with a concentration of 20% at 48 hours incubation, the inhibition zone decreased to 15.20 mm, which means it shows bacteristatic properties and only inhibits the growth of *E. coli* and is not lethal. Concentration of 10% incubation 48 hours, the inhibition zone formed decreased to 2.70 mm. According to Małgorzata Kikowska, et al. (2016), a 10% concentration of garlic has a very weak ability to inhibit the growth of *E.coli* because the inhibition zone formed is less than 5 mm. According to Huan Yuchen et al.(2020), the ability of an antimicrobial compound to inhibit or kill a bacterium depends on the concentration given. However, the garlic extract with concentrations of 20% and 30% remained bacteristatic. This is because gram-negative bacteria can produce enzymes that have the ability to deactivate the phytoconstituents and bioactive components of garlic extract, so that they are only inhibitory, not lethal. In addition, *E. coli* has a multilayer cell wall structure consisting of lipoproteins, phospholipid outer membranes, and lipopolysaccharides, causing the penetration of antibacterial substances into the bacterial cell wall is more difficult than that of gram-positive bacteria (I. Samuel Milleri and Nina R. Salama 2018). Meanwhile, chloramphenicol is a commercial antibiotic that is widely used as an antibacterial, which inhibits protein synthesis and is bacteriostatic (P.George Dinos, et al., 2016).

Garlic extract bioactivity test against Candida albicans

Table 1

Garlic extract bioactivity test against *Candida albicans*

No.	*Concentration of Compounds*	*Average of zone inhibition (mm)*	
		48 hours	*72 hours*
A	Garlic ethanol extract 30 ppm	19.40	20.10
B	Garlic ethanol extract 20 ppm	18.70	19.20
C	Garlic ethanol extract 10 ppm	10.80	10.40
D	Chloramphenicol (positive control)	19.60	20.00
E	Aquadest (negative control)	00.00	00.00

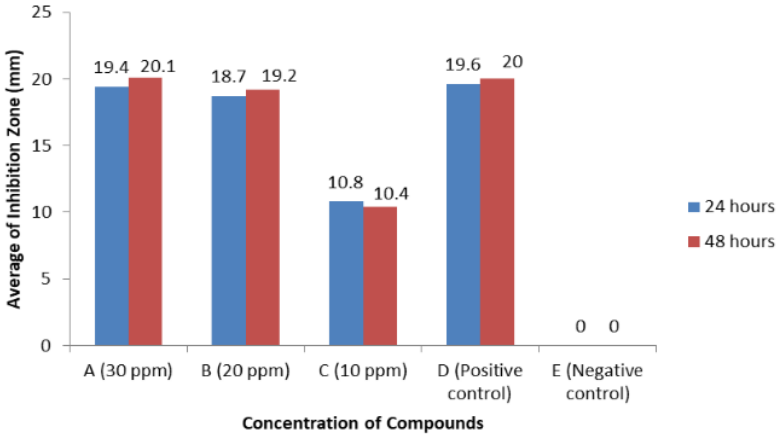

Figure 2. Histogram of inhibition diameter of garlic extract against the *Candida albicans.*

In Figure 2, it can be seen that the diameter of the largest inhibition at 48 hours of incubation consecutively was a concentration of 30% at 19.40 mm, a concentration of 20% at 18.70 mm, and a concentration of 10% at 10.80 mm. At 72 hours incubation the inhibition zone formed for 30% concentration increased to 20.10 mm and 20% concentration became 19.20 mm, while the 10% concentration decreased to 10.40 mm. Garlic extract concentrations of 30% and 20% were fungicidal against *Candida albicans*, while the 10% concentration was only fungicidal. According to Burian J. P, *et al*., (2017) garlic extract was proven to have fungistatic and fungicidal activity both in vivo and in vitro in inhibiting the growth of *C. albicans*. The ability of the active compound allicin from garlic as an anti-fungal has a role in inhibiting spore germination and hyphae growth in vivo and in vitro (Shang Ao, *et al.,* 2019). Allicin works to penetrate cell membranes, as well as mitochondrial organelle membranes, causing damage to organelles and causing cell death (Ding Guoliang, *et al.,* 2016). The results of the Scan of Electron Microscopy showed that *C. albicans* spores were destroyed and the only visible pseudohyphae were irregular (M. Tibor Nemeth, *et al.,*2018) and even damaged, this showed that garlic extract was able to kill the *Candida albicans*. (figure 3).

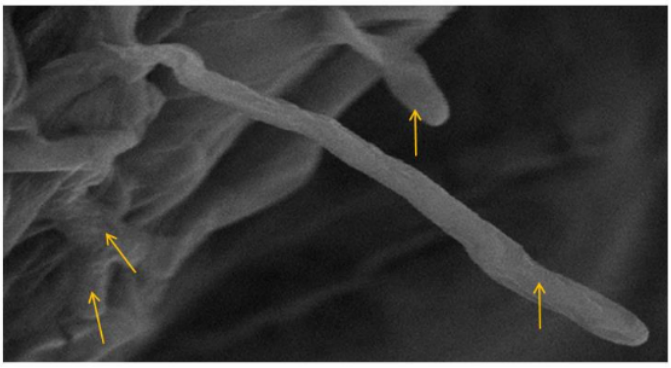

Figure 3. Results of Scan Electron Microscopy of Garlic extract against *C. albicans* (magnification 3000x).

Allicin is the active compound from garlic as an antimicrobial

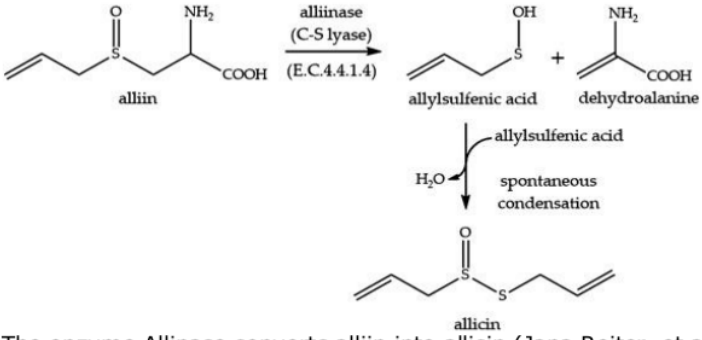

Figure 4. The enzyme Allinase converts alliin into allicin (Jana Reiter, et al., 2020).

Alliin is an organic sulfur compound contained in garlic which is synthesized from the amino acid cysteine by the enzyme allinase which is also present in garlic which will convert alliin into allicin when garlic is crushed or chopped (De Alberto Iseppi, et al., 2021). Allicin is an organosulfur compound that is responsible for antimicrobial activity by inhibiting RNA synthesis rapidly and thoroughly (Jana Reiter, et al., 2020). The way it works against bacteria and fungi according to Müller Alexandra, et al.(2016) is that allicin can inhibit bacterial growth by denaturing proteins and damaging cell membranes by dissolving fats found in cell walls. Allicin is able to migrate from the liquid phase to the fat phase so that damage to the cell membrane results in inhibition of the activity and biosynthesis of specific enzymes needed in metabolic reactions and causes death in bacteria (Ahmed Tanvir and Chin-Kun Wang. 2021). Allicin can also inhibit bacterial growth by inhibiting RNA production and lipid synthesis. This inhibition causes amino acids and proteins cannot be produced and the phospholipid bilayer of the cell wall cannot be formed, so that growth and development in bacteria will not occur (Aliashkevich Alena, et al., 2018). Singh R. N., et al. (2020) stated that allicin compounds increase the permeability of the bacterial wall which causes the SH groups (sulphihydryl and disulfide) to be destroyed in the amino acids cystine and cysteine. The destroyed SH group inhibits the synthesis of protease enzymes and damages the cytoplasmic membrane of bacteria, disrupting protein and nucleic acid metabolism, resulting in bacterial proliferation (F.James Zachary. 2017). The ability of the active compound allicin as antifungal by inhibiting spore germination and hyphae growth in vivo and in vitro (O. Fatimah Alothibi, et al., 2020). Allicin works to penetrate cell membranes, as well as mitochondrial organelle membranes, which causes damage to

organelles and causes cell death (Yang Xing Zhu and Yi-Rong Zeng. 2020). Allicin also induces and regulates proteins causing significant disturbances in normal cell metabolism and physical function of the fungus C. albicans (Yuan Yuan, et al., 2021).

Conclusions. The ethanolic extract of garlic has antimicrobial properties at concentrations of 20% and 30%, which are bacteriostatic against *E. coli* and fungicidal against the *C. albicans* at concentrations of 20% and 30%, respectively.

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Received: 23 August 2016. Accepted: 25 September 2016. Published online: 18 October 2016.
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How to cite this article:
Sutanto S., 2021 Potential of ethanolic garlic *Allium sativum* L. as an antimicrobial against *Escherichia coli* bacteria and *Candida albicans* fungus. AACL Bioflux 9(5):1090-1100.

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