International Baccalaureate

Biology Extended Essay

Comparative Investigation of Antibacterial Effects of Natural Products on *Escherichia Coli*

Research Question

To what extent do natural substances with known antibacterial properties such as vinegar (acetic acid), garlic (*Allium sativum*) and sage leaves (*Salvia officinalis*) differ according to their antibacterial effectiveness against *Escherichia coli* (ATCC 25922) as measured by the diameter of inhibition zones (±0.5mm) using the disk diffusion method under stable inoculum density, incubation conditions, disk size and extract volume?

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Introduction

The global antibiotic resistance crisis represents one of the most significant public health challenges of the 21st century, as it is becoming increasingly difficult to treat bacterial infections using conventional antibiotics. Growing up with parents who worked as microbiologists, I witnessed firsthand the scientific community's search for alternative antibacterial agents. Even though people have been using modern antibiotics for treatment for years, the number of the bacterial infections happening is increasing day by day.[1] So, I decided to do some research after hearing about this problem and I learned that plant-based substances can be used as an alternative to conventional antibiotics.[2] Therefore, my personal experience, combined with the urgent need for antimicrobial solutions, motivated me to examine bacteria and antibacterial properties of natural substances that could be used as potential alternatives to antibiotics.

This essay aims to examine the antibacterial properties of the plant-based products: garlic, sage leaves and vinegar.

[1] Lee Ventola, C. *The Antibiotic Resistance Crisis Part 1: Causes and Threats*. Vol. 40, no. 4, 2015. Accessed 27 Apr. 2025.
[2] Nascimento, Gislene G. F., et al. "Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic-Resistant Bacteria." *Brazilian Journal of Microbiology*, vol. 31, no. 4, Oct. 2000, https://doi.org/10.1590/s1517-83822000000400003. Accessed 27 Apr. 2025.

Background Information

Vinegar is composed of acetic acid (CH_3COOH), an organic acid that has both bacteriostatic and bactericidal properties. In previously done researches, it is proven that acetic acid can inhibit foodborne pathogens, including *E. coli.* (Entani et al., 1998) The mechanism involves undissociated acetic acid molecules passing through the bacterial cell membrane and then dissociating within the cytoplasm, which leads to a decrease in intracellular pH. This acidification disrupts enzyme functions, damages macromolecular structures, and interferes with ATP synthesis and ionic balance. These processed result in bacterial death. (McDonnell & Russell, 1999) In this study, the antibacterial properties of vinegar will be tested using the agar well diffusion method, which will enable the visualization of acetic acid's inhibitory zone against *E. coli*.[3] [4]

The antibacterial effects of garlic (*Allium sativum*) arise from allicin, which can easily react with important components of bacterial enzymes. (Ankri & Mirelman, 1999). When allicin reacts with these enzymes, it stops them from working properly. Studies show that this process stops the bacteria from performing essential life processes including energy production and damage repair. (Cavallito & Bailey, 1944) This creates oxidative stress inside the cell and eventually causes the bacteria to die. (Borek, 2001). These processes make garlic a very effective antibacterial substance against many different types of bacteria, including *E. coli*. In this investigation, the agar well diffusion method will be used to test this effect by measuring the clear inhibition zones around wells filled with garlic extract.[5] [6] [7]

[3] Entani, Etsuzo, et al. "Antibacterial Action of Vinegar against Food-Borne Pathogenic Bacteria Including Escherichia ColiO157:H7." *Journal of Food Protection*, vol. 61, no. 8, Aug. 1998, pp. 953–959, https://doi.org/10.4315/0362-028x-61.8.953. Accessed 27 Apr. 2025.

[4] McDonnell, G., & Russell, A. D. (1999). *Antiseptics and disinfectants: Activity, action, and resistance*. Clinical Microbiology Reviews, 12(1), 147–179. https://doi.org/10.1128/CMR.12.1.147 Accessed 11 Oct. 2025.

[5] Entani, Etsuzo, et al. "Antibacterial Action of Vinegar against Food-Borne Pathogenic Bacteria Including Escherichia ColiO157:H7." *Journal of Food Protection*, vol. 61, no. 8, Aug. 1998, pp. 953–959, https://doi.org/10.4315/0362-028x-61.8.953. Accessed 27 Apr. 2025.

The main sources of sage's *(Salvia officinalis)* antibacterial activity are rosmarinic acid, carnosic acid, and 1,8-cineole. These compounds act mainly on the bacterial cell membrane, increasing its permeability and causing leakage of essential intracellular materials such as ions, proteins, and nucleic acids. The disruption of membrane integrity also affects proton motive force and ATP synthesis, leading to energy depletion and cell death. While inhibitory effects of sage on gram-positive bacteria have been shown on previous studies, their effectiveness against gram-negative bacteria such as *E. coli* is often limited due to the protective outer membrane barrier (Ribeiro et al., 2015) (Bozin et al., 2007). Based on these established mechanisms of action, this investigation will assess sage's antibacterial activity through the agar well diffusion method.[8] [9] [10]

Bacteria are classified as Gram-positive and Gram-negative based on differences between their cell wall structures. Gram-positive bacteria consist of a thick peptidoglycan layer and do not have an outer membrane. The structure of Gram-positive bacterium allows many antibacterial agents to penetrate easily. Gram-negative bacteria are surrounded by a thin

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[6] Cavallito, C. J., & Bailey, J. H. (1944). *Allicin, the antibacterial principle of Allium sativum. I. Isolation, physical properties and antibacterial action*. Journal of the American Chemical Society, 66(11), 1950–1951. https://doi.org/10.1021/ja01239a048. Accessed 11 Oct. 2025.

[7] Borek, C. (2001). *Antioxidant health effects of aged garlic extract*. The Journal of Nutrition, 131(3s), 1010S–1015S. https://doi.org/10.1093/jn/131.3.1010S. Accessed 11 Oct. 2025.

[8] Yazgan, Hatice. "Investigation of Antimicrobial Properties of Sage Essential Oil and Its Nanoemulsion as Antimicrobial Agent." *LWT*, vol. 130, Aug. 2020, p. 109669, https://doi.org/10.1016/j.lwt.2020.109669. Accessed 27 Apr. 2025.

[9] Ribeiro, A. E., Amaral, C., Simões, M., & Martins, J. D. (2015). *Chemical composition and antibacterial activity of essential oils from aromatic and medicinal plants against foodborne pathogens*. Food Control, 54, 321–330. https://doi.org/10.1016/j.foodcont.2015.02.002 Accessed 11 Oct. 2025.

[10] Bozin, B., Mimica-Dukic, N., Simin, N., & Anackov, G. (2007). *Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils*. Journal of Agricultural and Food Chemistry, 54(5), 1822–1828. https://doi.org/10.1021/jf051922u. Accessed 11 Oct. 2025.

peptidoglycan layer as an additional barrier. This makes Gram-negative bacteria more resistant to plant-based antibacterial agents.[11] [12] [13]

E. coli is a gram-negative bacterium. It can cause a variety of illnesses such as pneumonia, bacteremia, peritonitis, and UTI when it is found outside of the digestive tract. [14] [15] Non-pathogenic *E. coli* strains provide the host nutrients such as vitamin K and B12. However, certain *E. coli* strains can cause diseases related to the gastrointestinal, urinary, or central nervous system.[16] In this experiment, the antibacterial effects of organic substances will be tested on a non-pathogenic *E. coli* strain due to its fast growth rate, simple culture, and great clinical microbiology relevance.

Hypothesis

If natural antibacterial substances made from garlic, vinegar, and sage leaf are applied to *E. coli*, then their inhibition zones will vary; with garlic expected to show the largest inhibition zone, followed by vinegar and sage. The inhibition zone diameters increase directly proportional with resistance to bacteria. Therefore, the expected order of antibacterial strength will be as garlic, vinegar and sage[17]

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[11] Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). *Brock Biology of Microorganisms*(15th ed.). Pearson Education. Accessed 13 Oct. 2025.

[12] Nikaido, H. (2003). *Molecular basis of bacterial outer membrane permeability revisited. Microbiology and Molecular Biology Reviews*, 67(4), 593–656. https://doi.org/10.1128/MMBR.67.4.593-656.2003. Accessed 13 Oct. 2025.

[13] Silhavy, T. J., Kahne, D., & Walker, S. (2010). *The bacterial cell envelope. Cold Spring Harbor Perspectives in Biology*, 2(5), a000414. Accessed 13 Oct. 2025.

[14] Kaper, James B., et al. "Pathogenic Escherichia Coli." *Nature Reviews Microbiology*, vol. 2, no. 2, Feb. 2004, pp. 123–140, https://doi.org/10.1038/nrmicro818. Accessed 27 Apr. 2025.

[15] Mueller, Matthew, and Christopher R. Tainter. "Escherichia Coli Infection." *PubMed*, StatPearls Publishing, 13 July 2023, www.ncbi.nlm.nih.gov/books/NBK564298/. Accessed 13 Oct. 2025.

[16] Nataro, James P., and James B. Kaper. "Diarrheagenic *Escherichia Coli*." *Clinical Microbiology Reviews*, vol. 11, no. 2, Apr. 1998, pp. 403–403, https://doi.org/10.1128/cmr.11.2.403. Accessed 27 Apr. 2025.

[17] "Agar Diffusion - an Overview | ScienceDirect Topics." *Www.sciencedirect.com*, 2016, www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/agar-diffusion. Accessed 27 Apr. 2025.

According to research, acetic acid, the main antibacterial component of vinegar, is usually weaker than the antibacterial effectiveness of allicin found in garlic (Murray, 2016) (Dean, 2012). On the other hand, the antibacterial effectiveness of phenolic compounds found in sage leaves is expected to be limited due to *E. coli* being a gram-negative bacterium and having a protective outer membrane. (Menderes et al., 2006)[18] [19] Therefore, I think the product with the largest inhibition zone diameter will be garlic.

Method Development and Planning

To ensure statistical reliability and account for experimental variability, five independent replicates were conducted for each test substance. This replication number was chosen because five trials are sufficient to facilitate meaningful statistical analysis and at the same time, it is not too many trials to conduct in a laboratory. The use of 3-5 replicates to determine consistent results and minimize resource usage is a common practice in previous studies of antimicrobial susceptibility testing (CLSI, 2020). The replicas were made up of individual agar plates containing all the test substances and controls and, therefore, inhibition zones could be measured independently. For antibacterial effectiveness testing, agar well diffusion method was selected since it is a standardized and simple technique for evaluating the antibacterial properties of plants and microbial extracts.[20]

Before starting the experiment, I made contact with researchers from the General Directorate of Public Health- Microbiology Reference Laboratory and Biological Products Department, as

[18] Dean, Sam. "Garlic Is as Good as One Hundred Antibiotics." *Bon Appétit*, May 2012, www.bonappetit.com/trends/article/garlic-is-as-good-as-one-hundred-antibiotics. Accessed 1 May 2025.
[19] Menderes, T, et al. *FARKLI YÖNTEMLERLE ELDE EDİLMİŞ SARIMSAK (Allium Sativum L.) EKSTRAKTLARININ ANTİMUTAGENİK ETKİLERİNİN ARAŞTIRILMASI HAZIRLAYAN: Mehmet Hakan SERMENLİ*. 2006 Accessed 1 May 2025.
[20] ScienceDirect. "Disk Diffusion - an Overview | ScienceDirect Topics." *Sciencedirect.com*, 2018, www.sciencedirect.com/topics/immunology-and-microbiology/disk-diffusion. Accessed 1 May 2025.

documented in Appendix 1. I received lab safety training and did my study under the supervision of Medical Microbiology Specialist Umut Berberoğlu.

Later, I examined previous researches on natural antibacterial agents to decide the most suitable natural products for my experiment. The accessible agents for me were vinegar, garlic and sage leaves.

For vinegar; we didn't have home-made vinegar at home so I bought grape vinegar from Fersan brand, containing 5% acetic acid.

For garlic, I found out that fresh garlic showed the most antibacterial properties so I used grocery-bought garlic that we already had in our house.[21]

Lastly, I used Bağdat brand store-bought sage leaves.

Figure 1: Garlic

Figure 2: Vinegar

Figure 3: Sage leaves

After obtaining all of my antibacterial agents, I kept all of them at room temperature until there was one day left until the beginning my experiment.

[21] Antibacterial and antifungal activities of spices. (2017). In *International Journal of Molecular Sciences* [Journal-article]. https://doi.org/10.3390/ijms18061283 Accessed 1 May 2025.

Preparation of Test Substances

- **Garlic Extract**

I peeled off 5g of garlic and weighed it using an analytical balance (±0.001g). Later, I crushed it and mixed it with 5.00±0.05mL of hot distilled water in a 50mL screw-cap polypropylene tube.[22] The mixture was Vortexed for 5±0.5 minutes using a digital vortex mixer and left at room temperature (~24°C) for 2 hours. For the final steps, the mixture was filtered through a sterile cloth into a 15±0.05mL glass centrifuge tube and stored in the refrigerator overnight (~4°C).

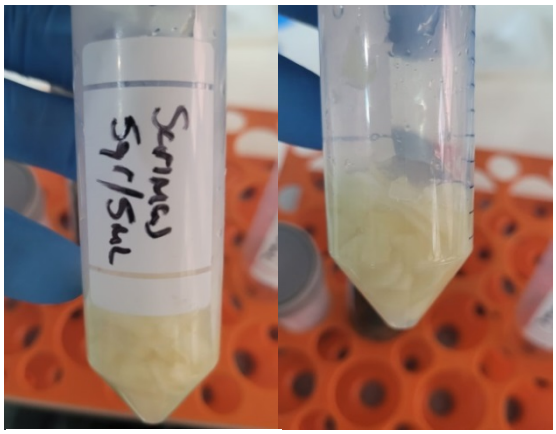


Figure 4: Garlic extract

Figure 5: 5.000 g garlic

- **Vinegar**

I used it directly without dilution. 10.00±0.05mL of vinegar was kept at room temperature in a 50.00±0.05mL polypropylene screw-cap tube and then transferred to a sterile tube and refrigerated overnight.[23]

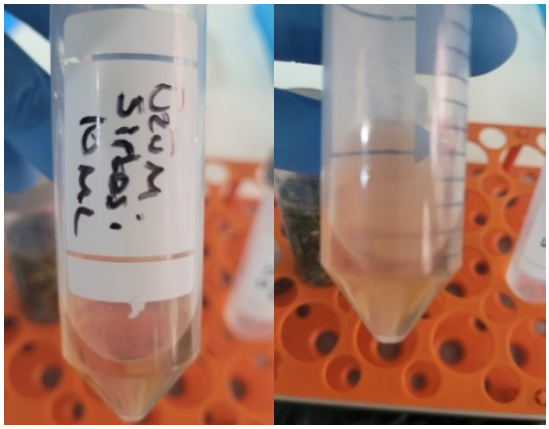


Figure 6: Vinegar

[22] Coşkun, F. & Namık Kemal Üniversitesi. (2010). Antimicrobial activity of some spices and spice extracts used in foods. *Akademik Gıda*, *8*(4), 41–46. Accessed 1 May 2025.
[23] Indu, M.N., et al. "Antimicrobial Activity of Some of the South-Indian Spices against Serotypes of Escherichia Coli, Salmonella, Listeria Monocytogenes and Aeromonas Hydrophila." *Brazilian Journal of Microbiology*, vol. 37, no. 2, June 2006, pp. 153–158,

- **Sage Suspension**

I weighed 5.014±0.001g of Bağdat brand dried sage leaves using an analytical balance (±0.001g) and mixed it with 10.00±0.05mL of hot distilled water.[24] The mixture was Vortexed for 5.0±0.5 minutes using digital vortex mixer and left at room temperature (~24°C) for 2 hours. Later, the mixture was filtered through a sterile cloth into a 15.00±0.05mL glass centrifuge tube and stored in the refrigerator overnight (~4°C).

Figure 7: Sage suspension

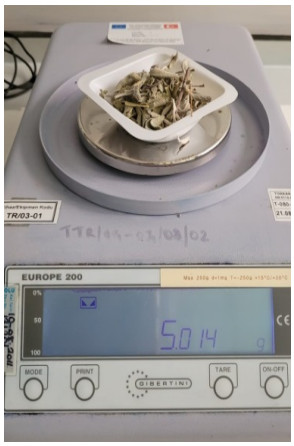


Figure 8: 5.014 g sage

- **Control Group**

I kept 10.00±0.05mL of distilled water at room temperature in a 50.00±0.05mL screw-cap tube and then transferred it to a sterile tube and refrigerated overnight.

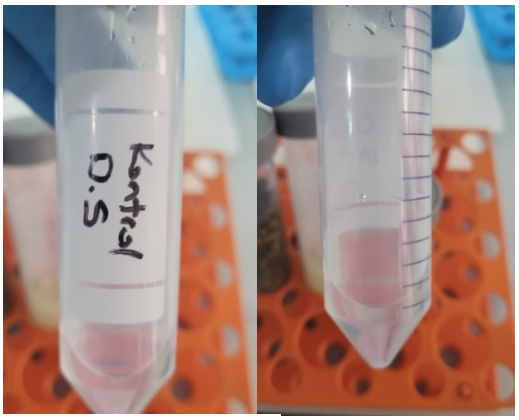


Figure 9: Control group

Figure 10: Test substances

www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-83822006000200011, https://doi.org/10.1590/s1517-83822006000200011. Accessed 1 May 2025.

[24] Ozkan, G. & Suleyman Demirel University. (2003). Inhibition of pathogenic bacteria by essential oils at different concentrations. *Food Science and Technology International*. https://doi.org/10.1177/1082013203009002003 Accessed 3 May 2025.

Hot distilled water was selected as the extraction solvent since organic solvents such as ethanol or methanol might offer higher extraction efficiencies for certain lipophilic compounds. Extraction of water offers a number of unique benefits to the study in the sense that it fits the theme of the study which is the exploration of natural, available alternatives to traditional antibiotics. The traditional medicine and domestic uses of water-based preparations are quite common. The solvent used to extract all the extracts should be the same so that there is no possibility of solvent-specific antibacterial effects that may confound comparisons between substances.

Also, hot distilled water effectively extracts water-soluble antibacterial components including allicin derivatives from garlic and phenolic acids from sage. This approach also maintains environmental compatibility and better simulates real-world conditions where these natural products would typically be prepared with water.[25]

Bacterial Strain and Culture Preparation

I did research about the correct *E coli* strain to use in my experiment. *Escherichia coli* ATCC 25922 and *Escherichia coli* ATCC 25923 bacterial strains were used in previous researches. (Nassan et al., 2015)[26] I asked for my supervisor about which strain was available in the laboratory. As a result, we decided that *Escherichia coli* ATCC 25922 bacterial strain was suitable for my study.

[25] Bae, Ji-Eun. "Hot-Water Infusion as an Efficient and Sustainable Extraction Approach for Edible Flower Teas." *Applied Sciences*, vol. 15, no. 23, 1 Dec. 2025, pp. 12730–12730, www.mdpi.com/2076-3417/15/23/12730, https://doi.org/10.3390/app152312730.

[26] Nassan, M., Mohamed, E., Abdelhafez, S., & Ismail, T. (2015). Effect of clove and cinnamon extracts on experimental model of acute hematogenous pyelonephritis in albino rats: Immunopathological and antimicrobial study. *International Journal of Immunopathology and Pharmacology*, *28*(1), 60–68. https://doi.org/10.1177/0394632015572075 Accessed 3 May 2025.

Although *E. coli* shows optimal growth at 37℃,[27] all incubation steps in this investigation were carried out at 24.0℃±1.0℃ to comply with the IB Ethics Policy, which restricts microbiological experiments to be done at temperatures below 25℃ to prevent the growth of potentially pathogenic bacteria. This lower incubation temperature ensured that bacterial activity remained at a safe, non-pathogenic level while still allowing sufficient growth for inhibition zone measurement.

Variables	**Name of Variable**	**How is it measured**	**Why is it being measured?**
Independent Variable	Type of test substance		
	○ Vinegar (acetic acid)	20.0±0.5μL of commercial grape vinegar was placed on 6.0±0.5mm agar wells	To test the antibacterial effect of vinegar against *E. coli* and compare with other substances.
	○ Garlic extract (allicin)	Prepared by mixing 5.000±0.001g of garlic with 5 mL hot distilled water, vortexed, filtered and refrigerated; 20.0±0.5μL placed per well.	To investigate the antibacterial effectiveness of allicin-containing garlic extract.
	○ Sage leaf suspension (rosmarinic acid)	5.000g±0.001 of dried sage mixed with 15mL hot distilled water, vortexed, filtered, refrigerated; 20.0±0.5μL applied per well.	To explore the inhibitory effect of sage's active compounds on *E. coli* growth.

[27] Doyle, M P, and J L Schoeni. "Survival and Growth Characteristics of Escherichia Coli Associated with Hemorrhagic Colitis." *Applied and Environmental Microbiology*, vol. 48, no. 4, 1984, pp. 855–856, https://doi.org/10.1128/aem.48.4.855-856.1984. Accessed 3 May 2025.

Dependent Variable	Diameter of inhibition zone around the agar wells (±0.5mm)	Measured using a ruler after 24 hours of incubation at 24.0±1.0℃.	Indicates the effectiveness of each substance in inhibiting *E. coli* growth.

Table 1: Independent and dependent variables explained

Variables	**Name of Variable**	**How is it measured**	**Why is it being measured?**
Controlled Variables	Bacterial strain (*Escherichia coli* ATCC 25922)	The same standard strain was used for all tests.	To ensure that differences in inhibition are due to the substances, not variability in bacterial type.
	Incubation temperature (±1.0℃) and time (hours)	All samples incubated at 24.0±1.0℃ for 24 hours.	Ensures consistent bacterial growth conditions across all trials.
	Growth medium (Tryptic Soy Agar)	All bacteria were cultured on the same type of agar.	Different media may influence bacterial growth, so using one type ensures consistency.
	Volume of substance applied to each disk (±0.5µL)	20.0±0.5µL applied to each well using a micropipette.	Standardizes the amount of antibacterial agent used in each trial.
	Agar well size (±0.5mm)	Sterile 6.0±0.5mm standard wells created.	Variations in size could affect the antibacterial activity.

	Turbidity of bacterial suspension ($\pm 1.5 \times 10^8$CFU/mL McFarland standards)	Adjusted to $0.5 \pm 1.5 \times 10^8$CFU/mL McFarland standards.	Standardizes bacterial concentration to avoid inconsistencies in growth.

Table 2: Controlled variables explained

Materials and Equipment	**Details/ Uncertainty**
Vinegar (grape vinegar)	Commercial brand (Fersan, 500 mL); 10.00±0.05mL used directly at room temperature
Garlic (*Allium sativum*)	Commercially bought; 5.000±0.001 g used with 5.00±0.05mL hot distilled water; vortexed 5 minutes; filtered and refrigerated
Sage leaves (*Salvia officinalis*)	Commercial brand (Bağdat, 40 g); 5.000±0.001g used with 15.00±0.05mL hot distilled water; filtered and refrigerated
Escherichia coli (E. coli) ATCC 25922	Non-pathogenic laboratory strain; cultured and adjusted to $0.5 \pm 1.5 \times 10^8$CFU/mL McFarland turbidity
Tryptic Soy Agar	Used as solid medium for bacterial culture (90.0x15.0±0.5 mm)
Saline peptone water	Used to prepare bacterial suspension or McFarland standard (500.00±0.05mL)
10-100 µL micropipette tip	Used to punch 6.0 mm holes in the agar; 5 holes per substance +1 control hole
Distilled water	Used in extraction and control disk
Digital vortex mixer	Used to mix the extract for 5.0minutes (±0.5minutes)

Incubator	PHCBI brand, MIR 154 model Refrigerated Incubator. Set at 24.0±1.0℃ for 24 hours
Pipette (adjustable)	For impregnating each disk with exactly 20.0µL of extract (±0.5µL)
Sterile forceps	For transferring disks onto Tryptic Soy Agar plate
Cotton-tipped swabs	Used to streak *E. coli* across Tryptic Soy Agar surface
Petri dishes/ Tryptic Soy Agar plates	One per substance. Total: 30 (90.0x15.0±0.5 mm)
Disposable loop	Used to transfer bacteria to saline peptone.
McFarland densitometer	BioSan brand. $\pm 1.5 \times 10^8$CFU/mL for standardizing bacterial density
Screw cap test tubes	75 tubes were used (10mL each)
Falcon tubes	Each is 50.00±0.05mL
Safety equipment	Lab coat, mask, medical gloves

Table 3: Materials and equipment explained

Method

- Passage the *E. Coli* strain onto pre-prepared Tryptic Soy Agar plates (prepared in 90x15 mm sterile polystyrene Petri dishes)
- Incubate it at 24.0±1.0℃ for 24 hours.
- Suspend the colonies in saline peptone water and vortex them.
- Adjust the bacterial suspension to $0.5 \pm 1.5 \times 10^8$CFU/mL McFarland standard turbidity.
- Take the extracts out of the fridge one hour before the experiment. Allow them to reach room temperature.

Figure 11: E. Coli strains passaged onto Tryptic Soy Agar plate

Figure 12: Colonies suspended in saline water

Figure 13: $0.5 \pm \mathbf{1.5} \times \mathbf{10^8}$ CFU/mL McFarland standard turbidity

- Spread the bacterial suspension evenly on 4 Tryptic Soy Agar plates using a sterile cotton swab.
- Allow plates to dry at room temperature for 10 minutes.
- Divide the plates into 6 sections. (prepare 5 sections for the trials and 1 section for a control group on each plate)
- Punch 6.00±0.05mm diameter wells into the agar using sterile tip of a 10-100µL micropipette tip. (1 well for each section)

- Pipette 20.00±0.05µL of each test extract into separate wells prepared for the test substances.
- Pipette 20.00±0.05µL of sterile distilled water into the wells prepared for the control groups.
- Incubate all plates at 24.0±1℃ for 18-24 hours in a calibrated incubator.
- After incubation, measure the diameters of the inhibition zones (clear areas around the disk where bacteria failed to grow) using a 30cm transparent metric ruler (±0.05mm).

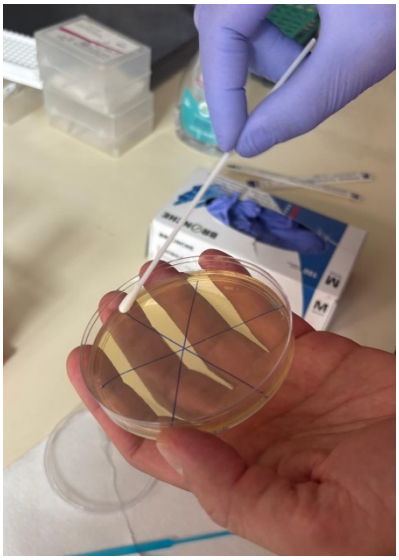


Figure 14: Bacterial suspension being spread on Tryptic Soy Agar plates

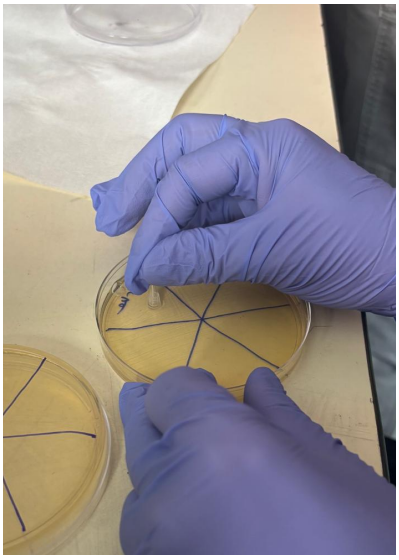


Figure 15: Process of punching wells into the agar

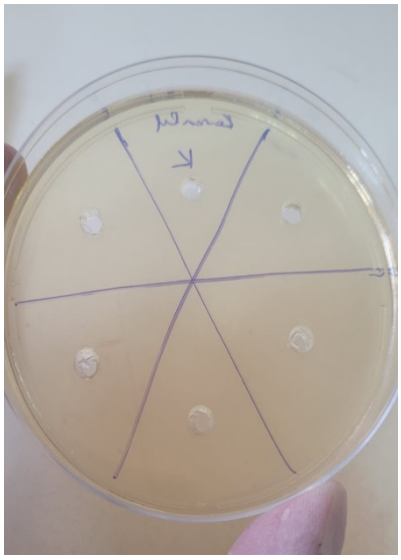


Figure 16: Agar wells first image

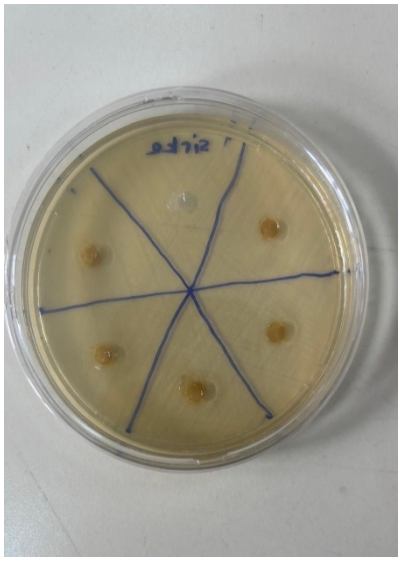


Figure 17: Sample of vinegar placed into prepared wells

Safety, Environmental and Ethical Concerns

Safety:

-All procedures were done in a controlled laboratory environment at the Microbiology Reference Laboratory, under the supervision of a trained microbiologist.

-Proper safety equipment was used including lab coats, gloves, safety gloves and mask; in order to minimize exposure to potentially harmful microorganisms.

Environmental:

-Naturally available products were used such as vinegar, garlic, clove and sage. They do not present any environmental hazards in the small quantities used.

-All biological waste was collected in designated containers and handled as biohazardous wastes. No active bacteria were released into the environment.

-All used bacterial cultures and agar plates were discarded following local laboratory biosafety and waste disposal regulations to ensure safety and environmental protection.

Ethical:

-The bacteria used in the study were non-pathogenic organisms and ethically acceptable for use in microbiological research. *E. coli* ATCC 25922 is a biosafety level 1 strain commonly used in laboratory testing. Although it is considered non-pathogenic under standard conditions, aseptic techniques were strictly followed. All bacterial cultures were handled under supervision and incubated below 25℃ in accordance with the IB Ethics Policy (IBO, 2018), ensuring no pathogenic bacterial growth.,

-No animal or human testing was involved.

-Permission was obtained from Microbiology Reference Laboratory, as documented in appendix 1.

Results

Garlic Extract:

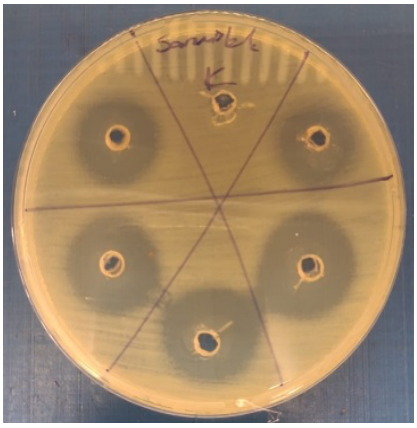


Figure 18: Garlic extract inhibition zones

Vinegar:

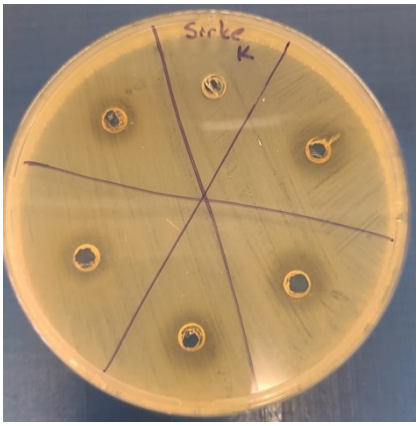


Figure 19: Vinegar inhibition zones

Sage Suspension:

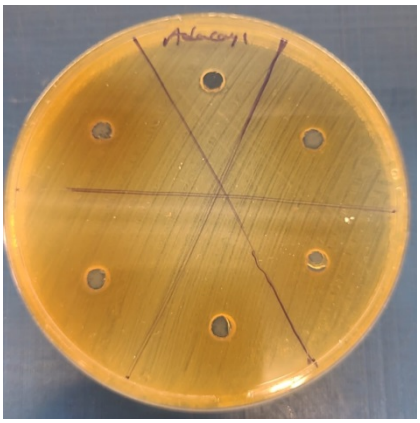


Figure 20: Sage suspension inhibition zones

Data Process

Raw Data Table

Test Substances	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Trial 4 (mm)	Trial 5 (mm)	Control Group (mm)	Uncertainty (± mm)
Garlic Extract	17.00	18.00	18.50	19.50	17.50	No zone	± 0.05
Vinegar	8.00	8.00	11.00	11.00	9.00	No zone	± 0.05
Sage Suspension	No zone	No zone	No zone	No zone	No zone	No zone	± 0.05

Table 4: Raw data table representing the diameters of inhibition zones obtained by each substance in each trial

Calculations

Mean zone:

$$Average\ zone\ diameter\ \ (\bar{x}) = \frac{sum\ of\ the\ diameters}{number\ of\ trials}$$

Sample calculation for garlic extract:

$$Average\ zone = \frac{17.00 + 18.00 + 18.50 + 19.50 + 17.50}{5} = 18.10\ mm$$

Standard Deviation:

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

s: the sample Standard Deviation

n: observation number

x_i: value of each observation

$\bar{x}$: the mean of samples

Sample calculation for garlic extract:

$$s = \sqrt{\frac{(17.0 - 18.1)^2 + (18.0 - 18.1)^2 + (18.5 - 18.1)^2 + (19.5 - 18.1)^2 + (17.5 - 18.1)^2}{4}}$$

$$= \sqrt{\frac{3.70}{4}} = \sqrt{0.925} \approx 0.96\ mm$$

For sage suspension, since there are no results, mean inhibition zone and standard deviation cannot be calculated.

Uncertainty Propagation:

$$\%\boldsymbol{uncertainty} = \frac{\boldsymbol{uncertainty}}{\boldsymbol{measurement}} \times \mathbf{100}$$

1. Percentage uncertainty connected with inhibition zone diameter measurements.

Sample calculation for mean garlic inhibition zone diameter:

$$\%\boldsymbol{uncertainty} = \frac{\mathbf{0.05}}{\mathbf{18.10}} \times \mathbf{100} \approx \mathbf{0.28}\%$$

2. Percentage uncertainty connected with the volume of applied extract.

$$\%uncertainty = \frac{0.50}{20} \times 100 = 2.50\%$$

3. Sum of all the percentage uncertainties:

Sample calculation for garlic percentage uncertainties:

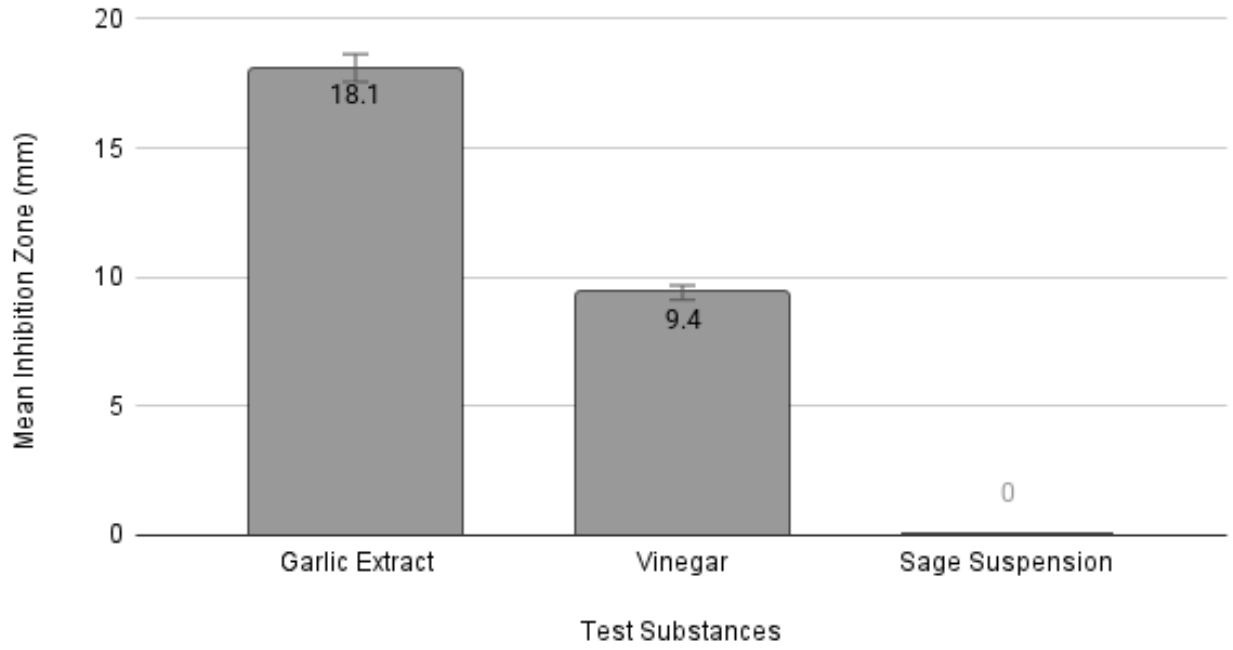
$$0.28\% + 2.50\% = 2.78\%$$

All these calculations are repeated for each test substance. Since there is no inhibition zone measured in sage suspension, only the percentage uncertainty connected with the volume of applied extract can be considered.

Processed Data

Test Substances	Mean Inhibition Zone (mm)	Percentage Uncertainty (%)	Standard Deviation (± mm)
Garlic Extract	18.10	2.78%	±0.96
Vinegar	9.40	3.03%	±1.52
Sage Suspension	No zone	2.50%	-

Table 5: Calculated mean inhibition zones, mean percentage uncertainties and standard deviations of each test substance



Graph 1: Mean calculated inhibition zones of each test substance with percentage uncertainties included

Statistical Analysis

Single Factor ANOVA was conducted using Google Sheets to examine if the mean inhibition zone diameters among three natural substances had statistically significant differences.

Source of Variation	Between Groups	Within Groups	Total
SS	819.43	12.90	832.33
df	2.00	12.00	14.00
MS	409.72	1.075	-
F	381.13	-	-
P-value	0.00	-	-
F critical	3.89	-	-

Table 6: Summary of Single Factor ANOVA results examining mean inhibition zone diameters among three natural substances and their statistically significant differences.

P-Value	P≤0.05	The evidence proves a relationship between variables.
	P>0.05	The evidence does not prove a relationship between variables.[28]
F and F crit Relationship	F>F critical	There is a significant difference between the values of different groups.
	F≤F critical	There is not a significant difference between the values of different groups.[29]

Table 7: P-value and F values ranges and definitions

According to the calculations done, the one-way ANOVA indicated a statistically significant difference between group means. To determine which pairs of group means differ, a Tukey's Honestly Significant Difference (Tukey HSD) post-hoc test was performed.

[28] Mcleod, Saul. "P-Value and Statistical Significance: What It Is & Why It Matters." *Simply Psychology*, 13 Oct. 2023, www.simplypsychology.org/p-value.html. Accessed 8 Oct 2025.
[29] Glen, Stephanie. "F Statistic / F Value: Definition and How to Run an F-Test." *Statistics How To*, 2022, www.statisticshowto.com/probability-and-statistics/f-statistic-value-test/. Accessed 8 Oct 2025.

Calculations

Standard error of the mean difference (SE)

$$SE = \sqrt{\frac{MS_{within}}{n}}$$

MS_{within}: mean square within groups from ANOVA

n: observation number per group

Test statistics for a pair

$$q = \frac{mean\ difference}{SE}$$

Sample calculation for garlic vs. vinegar significancy

$$MS_{within} = 1.075\ (from\ ANOVA)$$

$$n\ = 5\ (replicates\ per\ group)$$

$$Group\ means: Garlic = 18.1\ mm\ \&\ Vinegar = 9.4\ mm$$

$$Mean\ difference = 18.1 - 9.4 = 8.7\ mm$$

$$SE = \sqrt{\frac{1075}{5}} = \sqrt{0.215}\ \approx 0.4637$$

$$q = \frac{8.7}{0.4637}\ \approx 18.78$$

Comparison

The critical q value (q_{crit}=3.77) was determined from statistical tables for 3 groups and 12 degrees of freedom at the 0.05 significance level.

If $q > q_{crit}$, the pairwise difference is significant.

Since 18.78 > 3.77, the difference is statistically significant.

Comparison	**Mean difference (mm)**	q	**Comparison between q and q_{crit}**	**Significance**
Garlic- Vinegar	8.7	18.78	18.78 > 3.77	Significant
Garlic- Sage leaves	18.1	39.06	39.06 > 3.7	Significant
Vinegar- Sage leaves	9.4	20.28	20.28 > 3.77	Significant

Table 8: Summary of Tukey HSD post-hoc test results examining the statistical significance between pairs of groups of garlic, vinegar and sage leaves

The P-value was 0.00, and that is significantly lower than the generally accepted cut-off points of 0.05. F value (381.13) is greater than F critical value (3.89) and this proves that the differences are significant. A Tukey HSD post-hoc test was conducted to find whether there was a statistical significance between two test agents. The standard error was estimated to be about 0.464mm. Thus, all the pair-wise difference of mean values (Garlic-Vinegar=8.7mm; Garlic-Sage=18.1mm; Vinegar-Sage=9.4mm) were greater than 1.75 mm and it showed that the analysis was statistically significant.

The findings reinforce the hypothesis that the antibacterial effect of garlic extract is considerably higher than that of vinegar or sage. Given that sage leaves did not result in any measurable zones, it may be assumed that its antibacterial compounds were not effective in this experiment against E. coli.

The variance between the garlic and vinegar is also statistically significant and this fact validates that the mechanism of allicin against bacteria is far more effective than the antibacterial action of acetic acid in vinegar.

Conclusion

The agar well diffusion method was used to determine the antibacterial effect of vinegar, garlic extract and sage suspension against E. coli bacteria. Graph 1 illustrates that the greatest inhibition zones were produced by garlic extract, then vinegar and no visible antibacterial effects were observed on sage suspension. Garlic was the least error barred hence high credibility. Vinegar, however, had a slightly greater error bars probably because of its weak antibacterial activity.

The ANOVA and Tukey HSD tests indicated that the differences between the three substances were statistically significant, and the hypothesis that natural substances garlic, vinegar, and sage leaf treated to E. coli would exhibit different inhibition areas was accepted; as the most antibacterial activity should be exhibited by garlic, then vinegar, and then sage.

The mean diameter of the inhibition zone of the extract of garlic was measured to be 18.10 ± 0.05 mm and the standard deviation was 0.96mm; which is in line with the already available literature that allicin combines with critical bacterial enzymes resulting in cell death. Also, in earlier studies allicin was found to be a strong antibacterial compound against Gram-negative and Gram-positive bacteria (Ankri et al., 1999). (Cavallito et al. 1944) The high inhibition range of garlic in this experiment is congruent with other studies that have been conducted in the past.[30] [31]

Vinegar was found to have an average diameter of 9.40 ± 0.05 mm and a standard deviation of 1.52mm and exhibited smaller inhibition zone, which gave weaker, but quantifiable antibacterial activity. The finding of the experiment is in line with studies (Entani et al. 1998),

[30] Ankri, S., & Mirelman, D. (1999). *Antimicrobial properties of allicin from garlic*. Microbes and Infection, 1(2), 125–129. https://doi.org/10.1016/S1286-4579(99)80003-3 Accessed 12 Oct. 2025.

[31] Cavallito, C. J., & Bailey, J. H. (1944). *Allicin, the antibacterial principle of Allium sativum. I. Isolation, physical properties and antibacterial action*. Journal of the American Chemical Society, 66(11), 1950–1951. Accessed 12 Oct. 2025.

which indicate that acetic acid in vinegar has antibacterial properties against foodborne pathogens such as E. coli. The smaller inhibition areas than the garlic extract is due to the weak antibacterial power of acetic acid than that of allicin.[32]

Lack of inhibition of the sage extract could be due to the structural features of E. coli being a Gram-negative bacterium. The outer membrane of gram-negative bacteria serves as a shield against most of the hydrophobic molecules, including essential oils produced by plants (Nikaido, 2003). Consequently, the phenolic compounds in sage might not have been able to enter the bacterial cell wall. This observation is in line with the past literature that reported that the sage extracts are more active against Gram-positive bacteria compared to Gram-negative bacteria (Ribeiro et al., 2015).[33] [34]

In the control group, no inhibition zones were observed on all the trials, which proves the validity and sterility of the experimental setup.

The consistency of extraction yields is however a major methodological factor. Allicin is found in fresh garlic in a concentration of about 0.3-0.5% by weight, however, depending on its storage, age and mode of preparation. In the same way, sage phenolic content is subject to plant maturity, drying process and time of storage. Such natural fluctuations in raw materials, coupled with the inherent constraints of aqueous extraction add some degree of ambiguity to the absolute antibacterial effectivity that is being measured. It would be possible to use HPLC in future research to determine the precise concentration of antibacterial substances in each

[32] Entani, Etsuzo, et al. "Antibacterial Action of Vinegar against Food-Borne Pathogenic Bacteria Including Escherichia ColiO157:H7." *Journal of Food Protection*, vol. 61, no. 8, Aug. 1998, pp. 953–959, https://doi.org/10.4315/0362-028x-61.8.953. Accessed 27 Oct. 2025.

[33] "Molecular basis of bacterial outer membrane permeability revisited." *Microbiology and Molecular Biology Reviews*, 67(4), 593–656. Accessed 12 Oct. 2025.

[34] Ribeiro, A. E., Amaral, C., Simões, M., & Martins, J. D. (2015). *Chemical composition and antibacterial activity of essential oils from aromatic and medicinal plants against foodborne pathogens*. Food Control, 54, 321–330. https://doi.org/10.1016/j.foodcont.2015.02.002 Accessed 12 Oct. 2025.

extract. This would enable researchers to compare the effects of various concentrations on bacterial growth in a better way.

Moreover, in spite of the fact that this standardized method offers reproducible measurements of the inhibition zone, in clinical practice, antibacterial agents are exposed to complex biological structures, different pH, and competing microorganisms; all of which are not factored in this study. These shortcomings indicate that although the method is effective in the comparison of relative antibacterial strengths, its direct implication to the real world needs to be complemented by other studies like testing on food surfaces.

Also, the growth of E. coli at a temperature of 24°C instead of the ideal 37°C growth temperature attenuates the metabolism of the bacterium, which may not only influence the rate of growth but also the sensitivity to antibacterial agents. The low metabolic activity at 24°C can impact the fluidity of membranes and change the permeability of the membrane to such compounds as allicin and acetic acid. Also, slower growth may influence the size of the zones of inhibition as the diffusion rate is constant but the bacterial division rate is reduced. This difference in temperature probably had a bearing on the zone sizes, i.e. the relative efficacy of these natural substances at 37°C may vary. Future comparative studies with temperature control would be possible to measure this relation.

Evaluation

Strengths

- Three natural substances (garlic, vinegar, sage leaves) were subjected to the same set of standardized conditions.
- Every substance was run five times and statistically reliable results were obtained.
- The standardized bacterial suspension ($0.5\pm1.5\times10^8$CFU/mL, McFarland turbidity) and a well size were taken as constant.
- The incubation conditions were put under control (24.0±1℃ for 24 hours).
- The error analysis, standard deviation and error propagations were computed and examined with great care.

Limitations

Error	Explanation	Improvement
Manual measurement of inhibition zones with a ruler (±0.05mm)	Measuring inhibition zones manually with a ruler can cause human error, especially when the zone edges are not sharply defined.	Use digital image analysis software to measure inhibition zones accurately. This minimizes parallax error and provides objective data.
Simple extraction methods for natural products	Extraction by mixing samples in warm distilled water may not effectively release all active compounds.	Use ethanol-based extraction to obtain more concentrated extracts. This ensures active compounds are more soluble.
Single bacterial strain (*E. coli* ATCC 25922) used	Testing only one bacterial strain limits generalizability, as the results apply only to *E. coli* and not to other species or Gram-positive bacteria.	Include multiple bacterial strains like Gram-positive bacterium**.** It allows analysis of whether antibacterial effects are broad-spectrum or species-specific.

Manual agar well punching with micropipette tips (6.00±0.05 mm)	Manually punching wells can lead to slight variations in diameter or uneven depth, affecting diffusion rates of extracts.	Use a standardized agar puncher or metal cork borer to produce wells of uniform diameter and depth. This will improve the precision of the experiment and ensure consistent diffusion across each plate.
Wells placed in the same plate	Placing multiple wells in the same plate may lead to compound diffusion overlap, influencing the inhibition zone size.	Prepare individual plates for each trial. This prevents interference between samples and improves accuracy of inhibition measurements.
Pre-incubation room temperature not strictly controlled	Temperature fluctuations before incubation could affect bacterial growth rate and compound diffusion.	Use a temperature-controlled environment during pre-incubation such as a biosafety cabinet or incubator at constant 25±0.5 °C). Stable conditions improve reproducibility.
Only inhibition zone diameter measured	Measuring only zone diameters provides limited quantitative data on antibacterial activity. Diffusion rates and concentration gradients are not considered.	Complement the qualitative diffusion method with a MIC (Minimum Inhibitory Concentration) test using spectrophotometric analysis. This allows more quantitative comparison of antibacterial activity.

Table 9: Errors, Explanations and Improvements Explained

APPENDICES

Appendix 1: Public Health General Directorate of the Ministry of Health Letter of Confirmation

13 December 2024

Ayça Berberoğlu has conducted the experiment for her IB diploma programme extended essay by herself under the supervision of our staff in the microbiology laboratory of the Public Health General Directorate of the Ministry of Health.

Dr. Umut Berberoğlu
Medical Microbiology Specialist/PhD
Chief of National Parasitology Reference Laboratory

Appendix 2: Ethics and safety statement

General Directorate of Public Health – Microbiology Reference Laboratory and Biological Products Department

Microorganism Used: *Escherichia coli* ATCC 25922

Pathogenicity: *Non-pathogenic strain* used for laboratory testing of antimicrobial susceptibility (biosafety level 1 organism).

Security/Biosafety Code: BSL-1 (Biosafety Level 1)

Source: Obtained from the authorized culture collection of the General Directorate of Public Health's Microbiology Reference Laboratory.

This experiment complied fully with the IB Extended Essay Ethical Guidelines. No testing was conducted on humans, animals, or pathogenic microorganisms. The *E. coli* strain used is a non-pathogenic, biosafety level 1 reference strain, commonly used in antimicrobial research and safe for educational laboratories. All experiments were conducted under supervision of certified microbiologist Medical Microbiology Specialist Umut Berberoğlu. Appropriate personal protective equipment was worn at all times. All microbial materials and contaminated waste were autoclaved and disposed of according to biosafety regulations. No genetically modified organisms were used. No living animals and human subjects were used. All extracts of plants were obtained and prepared in a safe manner. The experiment did not do any harm to the environment and generated very little biodegradable waste.

Dr. Umut Berberoğlu

Medical Microbiology Specialist/PhD

Chief of National Parasitology Reference Laboratory