

**TED ANKARA COLLEGE FOUNDATION HIGH  
SCHOOL**

**Is there a significant mean difference between cooked and uncooked  
crushed garlic depending on the antibacterial effects?**

**Extended Essay (Biology)**

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## Abstract

The goal of this experiment is to observe the antibacterial properties of cooked and uncooked crushed garlic and the differences between cooked and uncooked crushed garlic.

My research question was : “ Is there a significant mean difference between cooked and uncooked crushed garlic depending on the antibacterial effects.”

A significant mean difference between cooked and uncooked garlic is expected as a result of this experiment. According to the antibacterial characteristics, the most efficient one will be the uncooked crushed garlic. On the other hand, the cooked crushed garlic will not have a significant bactericidal characteristic.

The Kirby-Bauer antibacterial testing method was preferred to conduct this experiment so as to test the hypothesis and to find the suitable answer for the research question. In laboratory conditions, incubation of *Escherichia coli* population was applied on Mueller-Hinton II agar plate. Filter paper disks were put onto the agar after being soaked in cooked and uncooked crushed garlic. The growth inhibition zone diameters were measured and by these diameters data analysis was done in order to investigate the differences between antibacterial characteristics of cooked and uncooked garlic.

As a result, the filter paper disk soaked in uncooked crushed garlic had the largest diameter of the growth inhibition zone. In contrast, cooked crushed garlic had nearly no significant growth inhibition zone around the filter paper disk. Results of T-test statistics showed that uncooked crushed garlic and cooked crushed garlic had an important mean difference in terms of their antibacterial effect on the tested microorganism named *Escherichia coli*, which is Gram negative bacteria.

## I. Introduction / Background

Garlic<sup>1</sup> which is called *Allium sativum* is included in the Alliaceae family and *Allium* genus. It is used not only as flavour in diet but also as cure for various diseases. The antimicrobial effect of garlic was first discovered by Louis Pasteur in 1858. By the latter discoveries, it is proved that garlic has antifungal, antibacterial and antiviral effects.

In history, breeding and consumption of *Allium* was first seen by Sumerians in Mesopotamia. Turkey contributes to the production of garlic by percent share of verse all over the world. Aegean Region, Black Sea Region, Mediterranean Region and Interior Anatolian Region are the most productive regions of garlic in Turkey.

Garlic which is used as nutrient supply and drug due to it's antimicrobial effect can be found in the forms of dry garlic, garlic powder, garlic puree, garlic oil and garlic capsules in the food and medical industry.

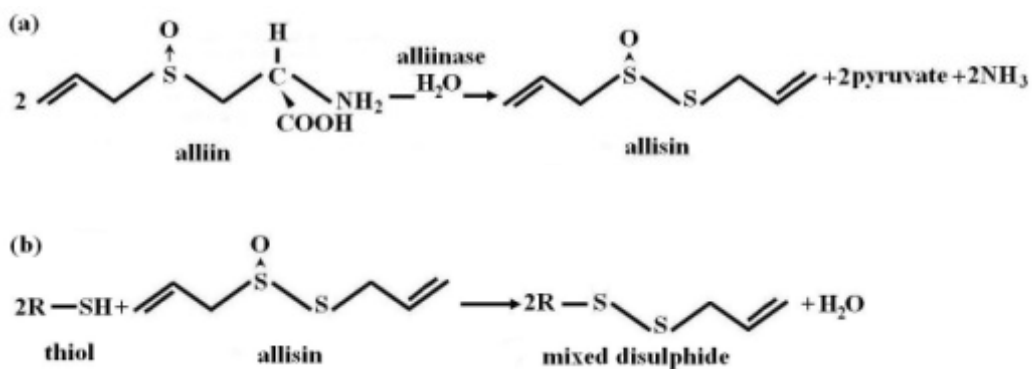
Ingredients of garlic are; proteins, fats, nitrogen containing compounds, minerals such as Magnesium, Iron, Potassium, Sodium, Zinc, Phosphorus and Manganate and vitamins such as Riboflovin, Niacin, Thiamin and Ascorbic Acid.

Garlic has antibacterial characteristic due to allisin compound that is isolated from diallyl thiosulphinade. Allisin compound also gives the sharp odor and the flavour of garlic. Garlic includes allisin compound in the range of 0.2-0.4% . Besides allisin there is another compound called alojen which also has antibacterial effect. Uncrushed garlic does not include allisin, whenever garlic is crushed, allisin is produced by allinase enzyme from alliin. Effect of the garlic is related to the reaction between the allisin and the –SH groups.

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<sup>1</sup>"Garlic." *Wikipedia, The Free Encyclopedia*. 6 Feb 2010, 20:05 UTC. 7 Feb 2010

<http://en.wikipedia.org/wiki/Garlic>



**Figure 1.**

a) Allisin synthesis from alliin

b) Thiol-disulphite change reaction between allisin and thiol

There is an allisin compound in the uncooked crushed garlic and the antibacterial characteristic is given to garlic by allisin. In other words, crushing the uncooked garlic activates the allisin compound and that's why uncooked crushed garlic has bactericidal and bacteriostatic effects. In contrast, cooked garlic does not include allisin compound to show antibacterial feature. Therefore, cooked form of garlic cannot show any bactericidal or bacteriostatic effect.

According to previous researches, the uncooked crushed garlic has broad spectrum by affecting both gram positive and gram negative bacteria. Moreover, Chloramphenicol which is the modern antibiotic has similar antibacterial effect with the uncooked garlic.

For many years, garlic is a cure for cardiovascular diseases. The levels of serum cholesterol and LDL cholesterol can be made lower by garlic. Blood pressure can also be decreased by garlic. In addition, certain types of cancer can be prevented and nervous system can be regulated by garlic. Infection can be prevented; nasal congestion and sinusitis can be improved again by garlic. Also one of the most significant biochemical characteristics of garlic is; it's antioxidant characteristic.

The bacterium *Escherichia coli*<sup>2</sup> was used in this experiment. It causes the infection of intestine which results with fever, stomachache and muscle pain. The reason why we chose this bacterium is that, it is taken by diet. Moreover, *Escherichia coli* is easily cultivated and incubated in laboratory conditions.

Bacterocidal term is used to define the agent which causes the death of bacteria population directly. Bacteriocidal agent generally damages the enzyme mechanisms of bacteria. On the other hand, bacteriostatic agent does not result in the death of the colony, it just blocks the replication of bacteria (Brittanica).

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<sup>2</sup>"Escherichia coli." *Wikipedia, The Free Encyclopedia*. Wikipedia, The Free Encyclopedia, 7 Feb. 2010. Web. 7 Feb. 2010. <[http://en.wikipedia.org/w/index.php?title=Escherichia\\_coli&oldid=3425320](http://en.wikipedia.org/w/index.php?title=Escherichia_coli&oldid=3425320)

## II. Hypothesis

The uncooked crushed garlic includes allinin which gives the antibacterial effect of garlic against some microorganisms; especially on *Escherichia coli*. Uncooked crushed garlic has both bactericidal and bacteriostatic effect due to the existence of allinin. The bacteriostatic effect of uncooked crushed garlic is both effective on Gram positive and Gram negative bacteria. *Escherichia coli* is an Gram negative bacterium whose growth is inhibited by uncooked crushed garlic extract. In other words, uncooked crushed garlic has bacteriostatic effects on Gram negative *Escherichia coli* bacterium. Moreover, uncooked crushed garlic has also bactericidal effect on *Escherichia coli*. It is proved that uncooked garlic reduce the number of viable cells which shows it's bactericidal characteristic.

The cooked crushed garlic do not include allisin<sup>3</sup> compound because allisin left the garlic due to heating process. Without allisin compound, garlic has no antibacterial characteristic. So that, cooked garlic has no bactericidal or bacteriostatic effect on microorganisms. In other words, cooked garlic do not reduce the amount of viable cells or it do not inhibit the growth of microorganisms.

According to this information, an important mean difference between cooked and uncooked garlic in terms of bactericidal and bacteriostatic effects is hypothesized. The uncooked garlic will have the highest effect of bactericidal and bacteriostatic features on *Escherichia coli*. On the other hand, cooked garlic will not have any significant bactericidal or bacteriostatic effects on *Escherichia coli*.

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<sup>3</sup>"Garlic." *Drug Information Online*. 009. Wolters Kluwer Health, Web. 7 Feb 2010. <<http://www.drugs.com/npc/garlic.html>>.

### III. Method Development and Planning

In this experiment, to compare the differences between cooked and uncooked crushed garlic and to observe the antibacterial effects of both groups, Kirby-Bauer method is preferred. The key point of this method is the diffusion principle. According to diffusion principle, highest concentration is found at the nearest point of the chemical. In other words, antibiotic which is still effective at low concentrations is the strongest one.

I have chosen this method since it is easy to set up the experiment, it gives quantitative results for data analysis and it has high accuracy levels.

In this method, the effect of antibiotic can be decided by looking at the inhibition zones (See Appendix 1) measured by the diameters of clear parts around filter papers. The clearness means that there is no bacteria production in this area due to the chemical agent. That's why I can decide the efficiency range of antibiotics by just looking at the diameters of these zones. Thus, I can compare the antibacterial differences of cooked and uncooked garlicks by measuring the inhibition zones around the filter paper discs.

Firstly, in this experiment, *Escherichia coli* is chosen to be used since it does not show any resistance to garlic. Then, I prepared the garlicks. I chose two garlicks at the same amount and same size. One of them was cooked and crushed and the other one was crushed without cooking. (They were crushed because allinase enzyme is active by crushing). Filter paper discs were prepared firstly by being sterilized at 121°C for 15 minutes. Then, sterilized filter paper discs were dipped in cooked and uncooked garlicks.

Mueller Hinton II agar<sup>4</sup> which is generally used in Kirby-Bauer method was used to culture *Escherichia coli* (shown at Figure2). It is known that agar has acid hydrolysate of casein, beef extract and starch to grow the bacteria at maximum level. Bacteria were cultured at 0.5 McFarland<sup>5</sup> which is the ideal value for Kirby-Bauer method.





Figure2: *Escherichia coli*

Agar is separated into two parts by marker to compare the differences of uncooked and cooked crushed garlics. Then, sterilized discs dipped in garlics were placed in different parts of petri plate.

Afterwards, these petri plates were incubated at  $36,5^{\circ}\text{C}$ , which is body temperature for humans, for 24 hours. After incubation inhibition zones were measured.

It is expected that the inhibition zone of uncooked crushed garlic will be greater than the cooked garlic.

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<sup>4</sup> <https://www.hardydiagnostics.com/catalog2/hugo/MuellerHintonMed.htm>

<sup>5</sup> The amount of initial bacteria planted to the agar is measured in McFarlands (See Appendix 2)

### **Materials used in the experiment:**

- 2 ml uncooked crushed garlic juice
- 2 ml cooked crushed garlic juice
- Petri dishes with Mueller-Hinton II nutritional agar in 4 x 90 mm size
- Filter papers in standard sizes
- *Escherichia coli* (non-antibiotic resistant bacteria)
- Distilled water
- Heraeus series 6000 Incubator
- Millimetric ruler (uncertainty: + or – 0.5 mm)
- Sterile knife
- 75% ethanol
- Zephiran

## **IV. Method**

Laboratory coat, gloves and mask must be worn during all procedure. The benches should be washed with Zephiran to prevent the contamination effect on the agar.

### **A. Preparation of Mueller-Hinton II nutritional agar:**

See Appendix 3.

### **B. Preparation of Antibacterial agent and filter papers:**

- By using distilled water, the bulbs of uncooked garlic were washed.
- By using sharp knife sterilized with 75% ethanol, the outer surface of uncooked garlic was removed.
- They were divided into small pieces, then crushed to activate allisin compound and to be used as the 1X dilution, it was mixed with 20 milliliters of water.
- From there, 1/4X and 1/16X serial dilutions were made.
- The steps which are mentioned above were repeated for cooked garlic.
- Standardized sterilized filter papers were embedded into cooked crushed and uncooked crushed garlic for 15 minutes to get the enough absorption of filter papers from garlicks.

### **C. Conduction of Experiment:**

- *E.coli* were cultured into the agar.
- Five filter papers were put in each the Mueller-Hinton II agar.
- The tops of the Petri dishes were shut.
- The petri dishes were put into the incubator for 24 hours at 37°C.
- Finally, the inhibition zones were measured and recorded.

## V. Results

The diameters of inhibition zones were measured to observe the antibacterial characteristic of cooked crushed and uncooked crushed garlic and the table below was prepared according this measurement.

Trials Type of Garlic	Zone of Diameters (+/- 1mm)				
	X1	X2	X3	X4	X5
Uncooked Crashed Garlic	19	20	20	21	18
Cooked Crashed Garlic	2	0	0	1	1

**Table 1:** Measurement of diameters of inhibition zones after 24h of incubation of *E.coli* population.

## VI. Data Analysis

The formulas<sup>6</sup> which are mentioned below are used to obtain the mean value, standard deviation and standard error.

### Mean:

$$\bar{X} = \frac{\sum_{i=1}^n X_i}{n}$$

where;

n is the trial number (n=5 for both groups)

X<sub>i</sub> for trial number i, the level of oxygen trapped in the graduated cylinder

### Standard Deviation:

$$\sigma = \sqrt{\frac{1}{N} \left( \left( \sum_{i=1}^N x_i^2 \right) - N\bar{x}^2 \right)} = \sqrt{\frac{1}{N} \left( \sum_{i=1}^N x_i^2 \right) - \bar{x}^2}$$

where;

n is the trial number (n=5 for both groups)

x<sub>i</sub> for trial number i, the level of oxygen trapped in the graduated cylinder

$\bar{x}$  is the mean value for the each group

### Standard Error:

$$SD_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

where;

n is trial number (n=5 for both groups)

$\bar{x}$  is the mean value for the each group

$\sigma$  is the standard deviation of the each group

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<sup>6</sup>Formulas are taken from the English version of Wikipedia

Groups	Count	Sum	Average	Variance	Standard Deviation
Uncooked crushed garlic impregnated filter papers	5	98	19.6	1.26	1.14
Cooked crushed garlic impregnated filter papers	5	4	0.8	0.7	0.84

**Table2:** The mean values, standard errors and standard deviations obtained by measuring the diameters of inhibition zones around filter papers which are soaked in uncooked crushed garlic and cooked crushed garlic.

### Hypothesis Testing<sup>7</sup>:

- 1) **Data:** They are mentioned in Table2.
- 2) **Assumption:** The data constitute two independent simple random samples each drawn from a normally distributed. The population variances are unknown but are assumed to be equal.
- 3) **Hypothesis:**  
 $H_0: \mu_c = \mu_u$  (The mean differences of uncooked and cooked crushed garlics are equal.)  
 $H_A: \mu_c \neq \mu_u$  (The mean differences of uncooked and cooked crushed garlics are not equal.)
- 4) **Test Statistics:** t-distribution is used for analysis

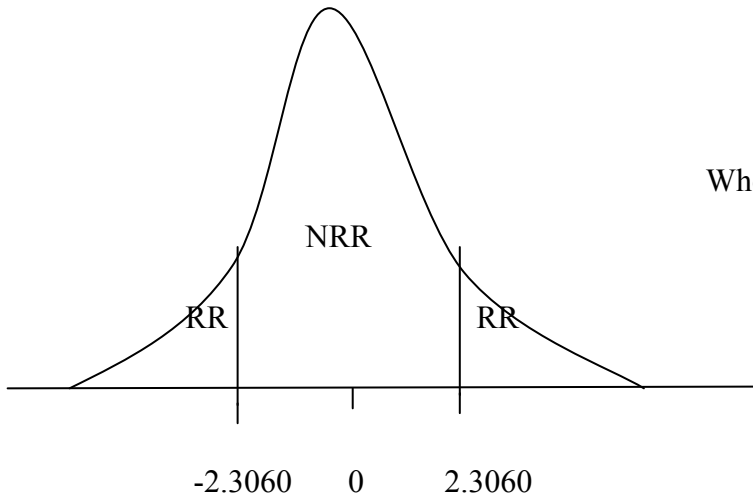
$$t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{\sqrt{\left(\frac{sp^2}{n_1}\right) + \left(\frac{sp^2}{n_2}\right)}} \quad \text{and} \quad sp^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$$

- 5) **Distribution of test statistics:**  $t$  distribution with  $(n_1 - 1)$  degrees of freedom is followed when the null hypothesis is correct.
- 6) **Decision Rule:** Let  $\alpha = 0.05$ . The critical value of  $t$  is 2.3060. Reject  $H_0$  unless  $t_{\text{computed}} > 2.3060$ .
- 7) **Calculation of Test Statistic:** From the sample data;

$$Sp^2 = \frac{4(1.14)^2 + 4(0.84)^2}{8} = 1.0026$$

$$t = \frac{(19.6 - 0.9) - 0}{\sqrt{\left(\frac{1.0026}{8} + \frac{1.0026}{8}\right)}} = 29.68$$

8) **Statistical Decision:** I reject  $H_0$ , since  $29.68 > 2.3060$ . It falls in the rejection region.



Where; RR: Rejection Region  
NRR: Non-rejection Region

9) **Conclusion:** In the light of these data, the means of cooked crashed garlic and uncooked crashed garlic are different from each other.

10) **P value:**  $p = 0.007$

- The null hypothesis should be rejected if the p value is less than or equal to  $\alpha$ ; if the p value is greater than  $\alpha$ , the null hypothesis should not be rejected.\*\*

Since  $p = 0.007 < 0.05$ , the null hypothesis is rejected. This means that there is a significant difference between cooked crushed garlic and uncooked crushed garlic.

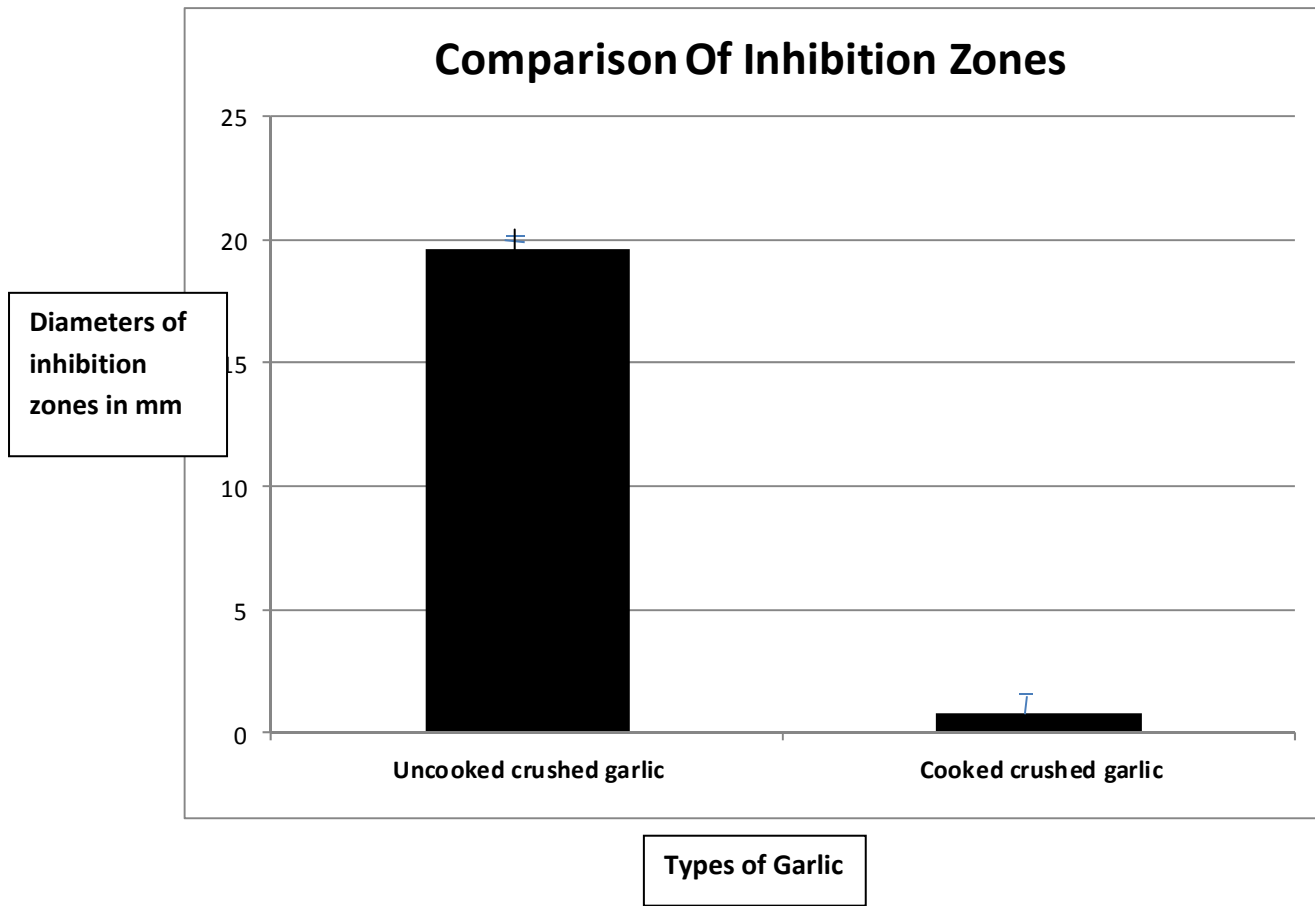
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<sup>7</sup> Minitab Statistical Programme is used for Hypothesis Testing

<b>Parameter</b>	<b>Uncooked Crushed Garlic</b>	<b>Cooked Crushed Garlic</b>
<b>Mean</b>	19.6	0.8
<b>Standard Error</b>	0.510	0.374
<b>Standard Deviation</b>	1.14	0.837
<b>Count</b>	5	5

**Table3:** This table shows the descriptive statistics for each experimental group. MINITAB statistical programme is used to get the data above.





**Graph 1:** The comparison of the inhibition zones obtained from uncooked crushed garlic and cooked crushed garlic. Standard errors for each groups are shown by error bars.

## VII. Evaluation

In this experiment, my goal was to determine the significant mean difference depending on the bactericidal characteristic between cooked crushed garlic and uncooked crushed garlic by using *Escherichia coli* in lab conditions. I conducted a hypothesis that there would be observed a significant mean difference in terms of bactericidal effects of both groups. Uncooked crushed garlic was expected to have the highest efficiency against *Escherichia coli*. On the other hand, cooked crushed garlic would have no significant bactericidal effect against *Escherichia coli*. Cooked crushed garlic showed the least antibacterial effect in my experimental design. The diameters of the inhibition zone diameters have a range between 0 to 2 mm with the mean value 0.8 mm. However, uncooked crushed garlic showed

highly more antibacterial effect than cooked crushed garlic by having the range of 18 to 21 mm and the mean value was measured to be 19.6 mm.

My null hypothesis was that; there was no significant mean difference between uncooked crushed garlic and cooked crushed garlic in terms of their inhibition zone diameters in Petri dish which was colonized with *Escherichia coli*. Due to pair-wise comparisons of both groups, calculated p-values were found to be smaller than 0.05, which lets me to reject my null hypothesis. Therefore, I concluded that; there is a significant mean difference between both groups in terms of bactericidal activity. (See Table2)

The results of the experiment and data analysis have supported my hypothesis which was " uncooked crushed garlic will have higher efficiency than cooked crushed garlic in terms of bactericidal property against *Escherichia coli* ". (See Table3)

In the Petri Dish that includes cooked crushed garlic was expected not to form any inhibition zone around the filter papers, however, I observed inhibition zones even in small diameters such as 1 or 2 mm. The reason why I got these unsuitable values may be that crushed garlic was not cooked properly so allisin, which is responsible for antibacterial effect of garlic, may remain in the structure of garlic. Conversely, in the Petri Dish that includes uncooked crushed garlic, I observed accurate values as it was expected but there was a small difference between the diameters. Non- uniformly colonized bacteria or non properly crushed garlic may be the reason for these differences. The standard deviation values of both groups are slightly different from each other (1.14 and 0.84). This occurrence is likely to be a random variation. In my experiment, there did not occur any unexpected situation that can be affect the results of the experiment. However, I will come up with some ideas which may affect the result of this type of experiments in an unexpected way:

- 1) The concentration of the garlic solutions. If they are used as over-diluted forms, their efficiency may be lower than the normal level. In contrast, if they are used in high concentration, their efficiency level

may be higher than the normal.

2) The absorption of the filter papers by the solutions. The duration of absorption by filter papers may be different in both trials.

3) Using only one type bacterium. Using other species may lead different results because of changes in resistance of different species. Actually, it is not an error for experiment, only I chose one type of bacterium in my experiment in order to limit the extend of this essay. Using other bacteria may give a chance to generalize the antibacterial characteristics of garlic.

4) The nutritional agar used in this experiment does not have the same features with the human body which is the best effective and suitable area for garlic to apply it's antibacterial features. However, there is no alternative nutritional agar similar to human body in order to use in Kirby-Bauer antibacterial testing method.

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<sup>8</sup> Hypothesis Testing with 2 paired  $t$  distribution

## VII. Conclusion

My research question : ‘‘ Is there a significant mean difference between cooked and uncooked crushed garlic depending on the antibacterial effects on *Escherichia coli* in laboratory conditions?’’ was answered with the help of the results of my study. There is an important difference in between their antibacterial efficiencies against *Escherichia coli* bacterium. Uncooked crushed garlic had a high efficiency as a bactericidal and bacteriostatic agent against *Escherichia coli* culture. On the contrary, cooked crushed garlic had nearly no efficiency as a bactericidal or even bacteriostatic agent against *Escherichia coli* culture.

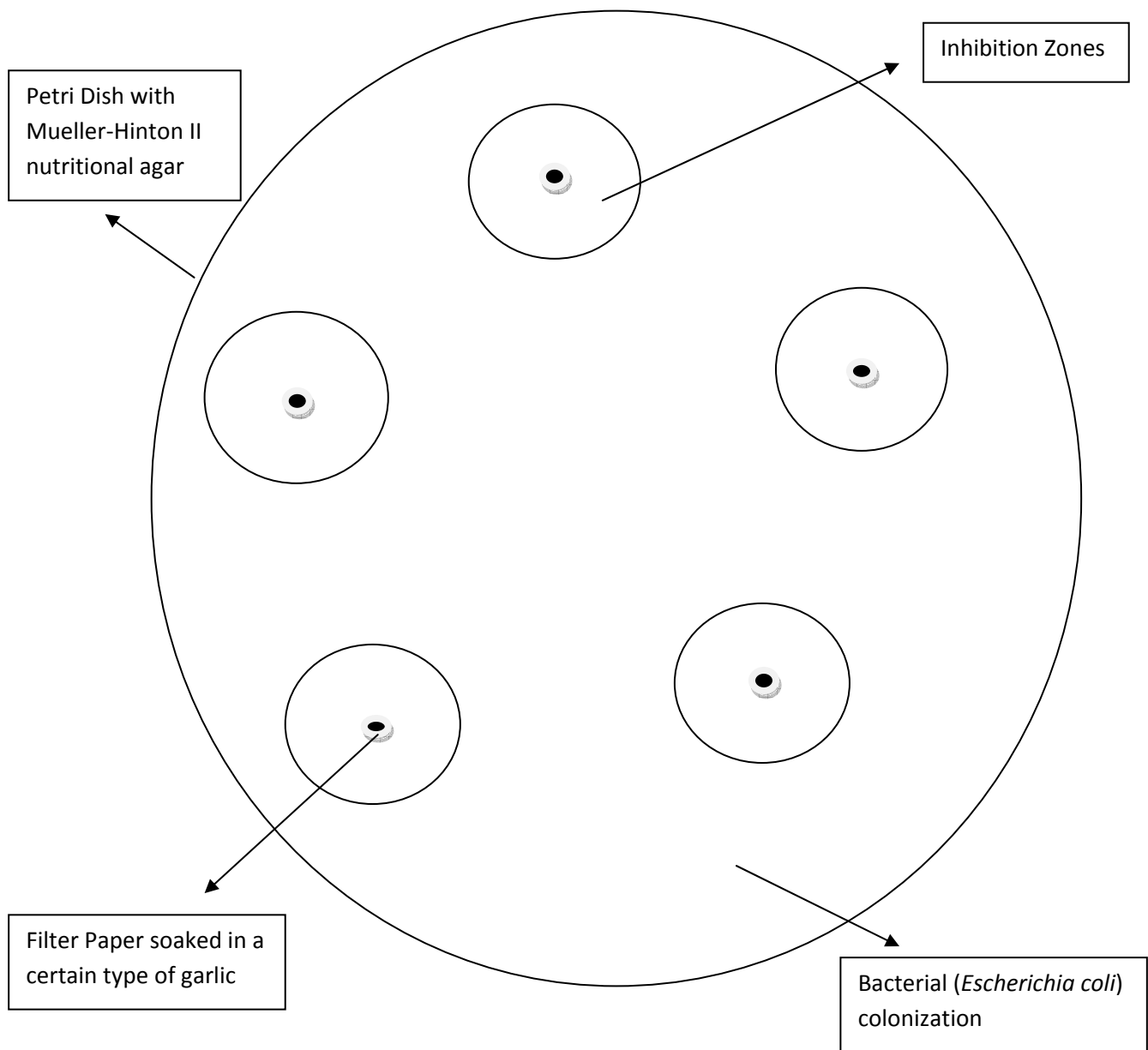
In this essay, a discussion was made about the antibacterial characteristics of uncooked and cooked crushed garlic and also comparison was made between these two groups depending on their bactericidal and bacteriostatic efficiencies. To limit the extent of

this essay, I applied the procedure to only *Escherichia coli* and made my conclusion according to the observations of the chosen bacterium.

Garlic has been consumed as food or used in the medical purposes since Sumerians. In the present day, garlic has an widespread usage and study area. Day by day, new products and medicals from garlic are going to be able for human usage. Garlic has an important place in human health due to its antibacterial and cancer arrester features.

# IX. Appendices

## a. Appendix 1



**Diagram 1:** The experimental design

**a. Appendix 2**

Information<sup>9</sup> which is given below is about McFarland standarts. McFarland standarts are applied to mention initial cell density in the nutritional agar.

**McFARLAND STANDARDS**

**PURPOSE:**

Usage of McFarland standarts aim the standardization of numbers of bacteria which provides laboratory guidance. Also another purpose of usage is susceptibility testing or other procedures that require a standardization of the inoculum. 0.5 McFarland standard is comparable to a bacterial suspension of  $10^8$  cfu/ml.

**PRINCIPLE:**

A standard inoculum of bacteria must be used 1for many types of susceptibility testing. McFarland standarts are constructed for two different aims. First aim is to replace the counting of individual cells and second aim is to match with approximate cell densities as required by the method of antimicrobial testing.

**FORMULAS:**

**0.5 McFarland Standard:** item no. R6540

- Sulfuric Acid, 1%.....995.00 ml
- Barium Chloride, 1%.....5.00 ml

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<sup>9</sup>The information is taken from “PML microbiological, Technical Data Sheet #500 Revision2”  
<http://www.pmlmicro.com/assets/TDS/500.pdf>

## **b. Appendix 3**

Below is information<sup>10</sup> for the Mueller-Hinton Agar, that is used as nutritional agar for E.coli bacterium in order to supply an environment for reproduction.

### **INTENDED USE**

Hardy Diagnostic Mueller Media has a significant usage in the cultivation of wide variety of microorganisms. Also Mueller Hinton Agar has an usage in the disk diffusion sensitivity testing of non-fastidious microorganisms. Furthermore, Mueller Hinton Broth is offered to prepare microorganism suspensions for disk diffusion sensitivity test.

### **SUMMARY**

Mueller Hinton Agar was proposed by Mueller and Hinton in 1941. Mueller Hinton Agar was produced to be a protein free medium in order to isolate pathogenic Neisseria strains. The effectiveness of Mueller Hinton Agar in identifying sulfonimide-resistant and responsive strains of gonococci was also founded by Mueller and Hinton. Recently Mueller Hinton Media has been started to be used in standardized antimicrobial disk susceptibility testing as Bauer mentioned, Kirby, et al. Barry and Fay were responsible for the investigation of the effects of altering the depth of plated Mueller Hinton Agar on disk diffusion testing, and with the help of their investigations they decided a standardized depth of nearly four millimeters to be sufficient. In 1970 Dewees, et al., researched the effect of storage on Mueller Hinton Agar plates used for antimicrobial disk diffusion zone sizes. The fact that, commercially manufactured Mueller Hinton Agar plates were appropriate for usage in routine susceptibility testing, was proved by their inventions. Additionally, the standards of Hardy Diagnostic Mueller Hinton Agar are determined by the Clinical laboratory Standards Institute.

Beef infusion, casamino acids and starch are the includings of the Mueller Hinton Media. Starch acts as colloid which gives protection to the medium against toxic material existing in the medium. Beef infusion

and casamino acids act as sources of energy and nutrient supply. If there occurs a need for solidifying agent, agar is added. The amounts of tetracycline and sulfonamide inhibitors, thymidine, thymine, magnesium and calcium ions are organized in way that do not effect susceptibilty testing and simultaneously yield satisfying growth. The Kirby-Bauer antimicrobial disk diffusion method is applied with Mueller Hinton Agar plates. This method is based on antimicrobial diffusion though an agar gel. The method requires soaked filter paper disk which is after placed on the agar surface. Zone diameters indicated for each of antimicrobial determining the resistant, intermediate and sensitive results for pathogenic microorganisms has been itimized in the Clinical Laboratory Standards Institute. Additionally, Mueller Hinton Broth's formulation is same with the Mueller Hinton Agar except the ingredient agar. Mueller Hinton Broth do not include agar. The main usage of Mueller Hinton Broth is for inoculation of microorganisms and in order to make dilutions of organisms which are to be used in the Kirby-Baeuer disk diffusion procedure.

#### **FORMULA**

Ingredients per liter of deionized water:

- Acid Hyrolysate of Casein 17.5 gm
- Beef Extract 2.0 gm
- Starch 1.5 gm

In addition to these, Mueller Hinton Agar contains:

- Agar 17.0 gm

Final pH 7.3 +/- 0.1 at 25 degrees C.

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<sup>10</sup> <https://www.hardydiagnostics.com/catalog2/hugo/MuellerHintonMed.htm>



## X. Bibliography

These references are written in MLA style.

Ayogu, T., and E. Amadi. "Studies On The Antibacterial Activities Of Medicinal Plants On Typhoid Fever Organism." *The Internet Journal of Third World Medicine*. 2009. Internet Scientific Publications, Web. 7 Feb 2010.

<[http://www.ispub.com/journal/the\\_internet\\_journal\\_of\\_third\\_world\\_medicine/volume\\_7\\_number\\_2\\_17/article/studies\\_on\\_the\\_antibacterial\\_activities\\_of\\_medicinal\\_plants\\_on\\_typhoid\\_fever\\_organism.html](http://www.ispub.com/journal/the_internet_journal_of_third_world_medicine/volume_7_number_2_17/article/studies_on_the_antibacterial_activities_of_medicinal_plants_on_typhoid_fever_organism.html)>.

"bacteriostatic." *Encyclopædia Britannica*. 2008. *Encyclopædia Britannica Online*. 01 Feb. 2010.

<<http://www.britannica.com/EBchecked/topic/48339/bacteriostatic>>.

Banerjee, Mousumi, and Prabir K. Sarkar. "Inhibitory effect of garlic on bacterial pathogens from spices." *World Journal of Microbiology and Biotechnology*. 19.6 (2003): 565-569. Print.

"Escherichia coli." *Wikipedia, The Free Encyclopedia*. Wikipedia, The Free Encyclopedia, 7 Feb. 2010. Web. 7 Feb. 2010. <[http://en.wikipedia.org/w/index.php?title=Escherichia\\_coli&oldid=342532052](http://en.wikipedia.org/w/index.php?title=Escherichia_coli&oldid=342532052)>.

"Garlic." *Drug Information Online*. 009. Wolters Kluwer Health, Web. 7 Feb 2010.

<<http://www.drugs.com/npc/garlic.html>>.

"Garlic." *Wikipedia, The Free Encyclopedia*. Wikipedia, The Free Encyclopedia, 6 Feb. 2010. Web. 7 Feb. 2010. <<http://en.wikipedia.org/w/index.php?title=Garlic&oldid=342258789>>.

Gaw, Dr. Betty. "Health&Wellness." *CHSSC.Health&Wellness*. 2006. Dr. Betty Gaw, Web. 7 Feb 2010. <<http://www.chssc.org/Wellness/HW0506.htm>>.

Maidment, D.C.J., Z. Dembny, and D.I. Watts. "Nutrition & Food Science." *anti-bacterial activity of 12 against*. 31.5 (2001): 238-241. Print.

"Mueller Hinton Media." Hardy Diagnostics: Your Microbiology Specialist. Hardy Diagnostics. 04 Feb. 2010. <<https://www.hardydiagnostics.com/catalog2/hugo/MuellerHintonMed.htm>>

Özçelik, Semra, Zeynep Sümer, Serpil Değerli, Fatih Ozan, and Atalay Sökmen. "Sarımsak(*Allium Sativum*) Özütü Skolosidal Ajan Olarak Kullanılabilir Mi?" *Türkiye Parazitoloji Dergisi*. 31.4 (2007): 318-321. Print.

"PML Microbiologicals". Jan.2001. PML Microbiologicals. 03 Feb. 2010

<<http://www.pmlmicro.com/assets/TDS/500.pdf>>.

Queen's University. "Chemists Shed Light On Health Benefits Of Garlic." *ScienceDaily* 31 January 2009.

7 February 2010 <<http://www.sciencedaily.com/releases/2009/01/090130154901.htm>>