INTERNATIONAL BACCAULARATE PROGRAMME

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EXTENDED ESSAY

(BIOLOGY)

"INVESTIGATION OF ANTIBACTERIAL EFFECTS OF ONION JUICE, HONEY AND ONION-HONEY COUGH CURE ON *E. COLI* AND *S. AUREUS*"

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RESEARCH QUESTION: “How do different concentrations of onion-honey cough cure affect the gram positive bacteria, *S. aureus*, and gram negative bacteria, *E. coli*, compared to onion juice and honey extract?”

ABSTRACT

Since the ancient times, onion and honey is used to cure several diseases, mostly because of their antibacterial properties. Onion-honey cough cure is a remedy prepared with onion juice and honey and it is used to alleviate cough and the symptoms of cold. Although this remedy is used a lot in Turkey, it is not certain whether the onion-honey cough cure is more effective against the bacteria compared to only onion juice or only honey. Because of that, the aim of this experiment was to investigate the antibacterial effects of onion juice, honey and onion-honey cough cure for their different concentrations. Since the resistivity of gram-positive and gram-negative bacteria towards antibacterial agents are different, antibacterial effects of onion-honey cough cure, onion juice and honey investigated on a gram-negative bacterium, *E. coli*, and a gram-positive bacterium, *S. aureus* separately. Microtiter plates were filled with different concentrations of onion juice, honey and onion-honey cough cure and they were inoculated with *S. aureus* and *E. coli* with the help of Micro Dilution Test.

It was found that since onion-honey cough cure has the same antibacterial property with the flower honey for *E. coli* (reproduction of *E. coli* both starts at a concentration of 50%) and it has the same antibacterial property with onion juice for *S. aureus* (reproduction of *S. aureus* both starts at a concentration of 6.25%), it cannot be accepted that onion-honey cough cure has a greater antibacterial effect compared to onion juice and flower honey. Besides that, it was seen that *S. aureus* was more susceptible to onion juice, honey and onion-honey cough cure compared to *E. coli* when the concentration they start to reproduce is compared. On the other hand, difference between the antibacterial effects of onion juice, honey and onion-honey cough cure and the difference between the susceptibility of *E. coli* and *S. aureus* cannot be proven statistically since the p values of Chi-square test was always bigger than 0.05.

To sum up, results of the experiment showed that onion juice, honey and onion-honey cough cure have all antibacterial properties on the gram positive bacteria, *S. aureus*, and gram negative bacteria, *E. coli* but the difference between their effectiveness cannot be proved with Chi-square test due to unbalanced distribution of the data.

INTRODUCTION

When I was a little girl, I was constantly getting ill and I had to use antibiotics for long periods. Since my grandmother was worried about the side effects of the antibiotics, she was looking for alternative remedies such as onion-honey cough cure. Until last year, I didn't think that this remedy can be beneficial but then, I saw that my grandfather started to cough rarely after he used this cure. This issue has attracted my attention so I decided to make some research about it.

I was surprised to find that the ancient Egyptians, Chinese, Greeks and Romans employed honey for wounds and acute fever. Modern researches show that honey at a concentration of 40% is bactericidal to various gram-negative and bacteria.[1] Antibacterial properties of honey are due to its osmotic pressure, pH and the H_2O_2. All types of honey have high sugar content but a low water content and acidity, which prevent microbial growth.[2] Onion was also used for treatment in ancient times. In Arabic countries, it was usually used as a treatment for eye diseases, worms, skin rashes. The Greek physician Hippocrates recommended it for pneumonia. Pasteur was the first researcher to describe antibacterial effect of onion and garlic and many health benefits of onion are attributed to high amount of organosulphur compounds.[3]

As it is indicated above, onion and honey extracts can have bactericidal effect on gram positive and negative bacteria. So, I started to make a research to find one gram positive and one gram negative bacteria that I can use in my experiment and found two suitable kinds. First one is the *Escherichia coli*, a gram negative rod. The optimal temperature for its growth is 37 °C. It is a common inhabitant of the intestinal tract of man and most strains of *E. coli* are harmless.[4] Its growth conditions and place of inhabitancy makes it a suitable choice. Second one is *the Staphylococcus aureus*, a gram positive bacterium. Bacteriological culture of the nose, mucous membrane and skin of humans yield staphylococci.[5] Spectrum of disease caused by *S. aureus* ranges from mild skin infections to serious disease like pneumonia and.[6]

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[1] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1292197/?page=1

[2] http://www.biomedcentral.com/1472-6882/9/34/

[3] http://archiv.ub.uni-marburg.de/diss/z2007/0404/pdf/djj.pdf

[4] http://www.hi-tm.com/1908/SECTION-2-D-1908.pdf

[5] http://textbookofbacteriology.net/staph.html

[6] http://www.livestrong.com/article/83622-characteristics-staphylococcus-aureus/

Since honey was used to cure wounds and onion was used to cure pneumonia in ancient times, I thought that *S. aureus* is a good choice.

Since both onion and honey have antibacterial properties, it is inevitable that onion-honey cough cure also has this property but according to an article in "The Times", it is not certain whether the active component is the onion, honey or combination of them.[7] Besides that, I wonder the active substances, onion juice, honey and onion-honey cough cure, is more effective on a gram positive or gram negative bacteria. Because of that in my experiment I will focus on the research question "How do different concentrations of onion-honey cough cure affect the gram positive bacteria, *S. aureus*, and gram negative bacteria, *E. coli*, compared to onion juice and honey extract?" and I will discuss the method of the experiment and analyze the data obtained.

[7] http://apitherapy.blogspot.com/2006/01/honey-and-onion-cough-cure.html

HYPOTHESIS

Different researches and articles show that the onion juice and honey have antibacterial effects. In his article, Sampath Kumar et al state that onion contains thiosulphate, a compound that is effective in killing many common bacteria, including *Escherichia coli*.[8] In addition to that, Al Masaudi and Al Bureikan write that the onion juice, honey and onion-honey cough cure have inhibitory effects separately on both Gram-positive (*S. pyogenes*, *S. aureus*, *Enterococci*)and Gram-negative (*E. coli*, *P. aeruginosa*) bacteria. Their research showed that the combination of onion-honey has stronger growth reduction activity on microorganisms than onion alone or honey alone due to the acting alone or synergistically of the elements. In addition to that, *S. aureus* was more sensitive to honey compared to *E. coli* while *E. coli* was more sensitive to onion juice and onion- honey cough cure.[9]

Gram positive and negative bacteria differ from each other by their cell wall structures. In most gram-positive bacteria, the cell wall consists of many layers of peptidoglycan while the gram-negative bacteria consist of a very few layers of peptidoglycan and an outer membrane consist of lipoproteins. One of the specialized functions of the outer cell of the gram-negative bacteria is to provide a barrier to certain antibiotics and digestive enzymes. (Tortora & Funke & Case, 78)[10]

I expect that the active substances will affect both *S. aureus* and *E. coli* but considering the information given above, it can be hypothesized that the onion-honey cough cure will have a greater antibacterial effect on *S. aureus* and *E. coli* since onion-honey cough cure includes antibacterial substances from both honey and onion juice and the gram negative bacteria, *E. coli*, will be less affected compared to the gram-positive bacteria, *S. aureus* due to their lipoprotein cell walls.

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[8] http://jocpr.com/vol3-iss6-2011/JCPR-2011-3-6-997-1003.pdf

[9] "Antimicrobial Activity of Onion Juice (Allium cepa), Honey, and Onion-Honey Mixture on Some Sensitive and Multi-Resistant Microorganisims "

[10] Tortora, Gerard J. and Funke, Berdell R. and Case, Christine L. (1995) Microbiology An Introduction (Fifth Edition), Canada: The Benjamin/Cummings Publishing Company

METHOD DEVELOPMENT AND PLANNING

I carried out my experiment in the laboratory of Microbiology Department of the Başkent University with the help of Prof. Dr. Jülide Sedef Göçmen. Designing the most suitable method to investigate the hypothesis has brought several problems. The first problem was to decide on what kind of onion and honey I will use. I chose red onion because more juice can be extracted from this kind of onion compared to white onions. Choosing the most suitable kind of honey was more complicated. I bought flower and pine honey and I looked for a suitable method to sterilize them but I couldn't find any. So, I decided to investigate whether there are any bacteria in these different honeys. In two separate petri dishes, I transferred the appropriately diluted flower and pine honey to agar plate and I left the petri dishes in the incubator for a day. When I check the dishes in the second day, I saw bacterial growth in the petri dish with pine honey, while the other one was clear. So, I decided to use the flower honey.

The second problem was to choose a suitable antimicrobial susceptibility test. My first option was Disc Diffusion Test Susceptibility Test. In Disc Diffusion Test antibiotic discs are placed in a petri dish inoculated with bacteria where they are allowed to diffuse into the growth medium to test the susceptibility of bacteria to that antibiotic. As the antibiotic diffuses into the growth medium, the bacteria start to reproduce. After a certain time of incubation at 35 °C, a zone can be formed around the disc according to the inhibition concentration of the antibiotic on the bacteria. After measuring the length of the zone of inhibition, the susceptibility of the bacteria can be understood by comparing that length to the standard zone lengths. My second option was the Micro Dilution Test. First of all, the antimicrobial agent is prepared in liquid culture medium (cation-adjusted Mueller Hinton II Broth is preferred) and it is diluted by a factor of two in the microplates. Same amount of microorganisms are added to each cell of the microplate filled with different concentrations of the antimicrobial agent. Also, a control group is formed by inoculating the microorganisms in a cell with growth medium that does not contain any antimicrobial agent. After a certain time of incubation at 35 °C, the turbidity of each cell is observed to see whether the microorganisms reproduce or not. I chose the second method because it was easier to prepare different concentrations of antimicrobial agents and it was easier to observe since one microplate contains all of the different concentrations.[11]

[11] www.klimik.org.tr/wp-content/uploads/2012/02/128201112107-49.pdf

While applying the Micro Dilution Test, I had to choose a suitable growth medium that will enhance the growth of the microorganisms and will be used to produce different concentrations of onion juice, honey and onion- honey cough cure. I thought the Mueller Hinton II Broth will be the best choice because it can be used for quantitative susceptibility testing of gram-negative and gram-positive aerobic bacteria.[12] Besides that, it is low in inhibitors and a large body of data and experience has been collected concerning susceptibility tests performed with this medium. The use of media other than this agar may result in erroneous results. (For the reagents, see Appendix 1)[13]

Making a decision on the method that will be used has not ended the problems. The flower honey that will be used in the experiment was too dense to be transferred by the multi channel automatic pipette. Because of that, although I was planning to start my investigation about the antibacterial effect of flower honey with 100% honey, I had to start it with 50% honey-Mueller Hinton II Broth mixture.

Another problem for me was the sterilization of the onion. On the first day of my experiment, I extracted the juice from the onion that was just rinsed with pure water. Then, I cultivated *E. coli* and *S. aureus* as outlined in the Method. When I return the lab to take the results, I saw that a kind of bacteria that I haven't used in the experiment has grown in the microtiter plates. It wasn't hard to understand this difference because the bacteria that had grown in sample wells caused a greenish color while *S. aureus* and *E. coli* can produce semi-transparent white color. Considering the fact that the apparatuses used in the experiment was carefully sterilized, we thought that the unexpected contamination of the microplates could be due to two factors: possible contamination of Mueller Hinton II Broth or the contamination of onion juice. Because of that, I decided to repeat the experiment with a new sterilization method and a new sample of Mueller Hinton II Broth. This time, I applied the sterilization method given in the article of C. Azu and A. Onyeagba and I used ethanol to sterilize the onion.[14]

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[12] http://www.bd.com/ds/technicalCenter/inserts/L007475(12)(201102).pdf

[13] http://www.microbelibrary.org/component/resource/laboratory-test/3189-kirby-bauer-disk-diffusion-susceptibility-test-protocol

[14] "Antimicrobial Properties of Extracts of *Allium cepa* (Onions) and *Zingibar officinale* (Ginger) on *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*"

It is important to keep some variables constant to obtain more accurate results like temperature for incubation, amount of the bacteria in each sample well and using the same strain of the *S. aureus* and *E. coli*. Microtiter plates should be kept in a constant temperature since it can affect the growth of the bacteria. Because of that, they were left in the incubator adjusted to 37 ºC, the most suitable temperature for the rapid growth of bacteria, for 24 hours. The same standard strain of *E. coli* and *S. aureus* were used in each trial because the resistivity of each bacterium towards the medicines and external factors should be similar. For my investigation, I used *E. Coli* ATCC 25922 and *S. aureus* ATCC 25923 standard strains. The amount of the bacteria in each sample well should be constant because if the amount of the bacteria in one sample well increases, their chance to survive in that medium also increases. While preparing the solutions of bacteria, I used saline because it does not change the properties of the bacteria and affect its reproduction.

As indicated above, the first few trials were not successful because of the problems with sterilization but that fail helped me to improve the weaknesses of the experiment and have more accurate results in the other trials.

DIAGRAMS

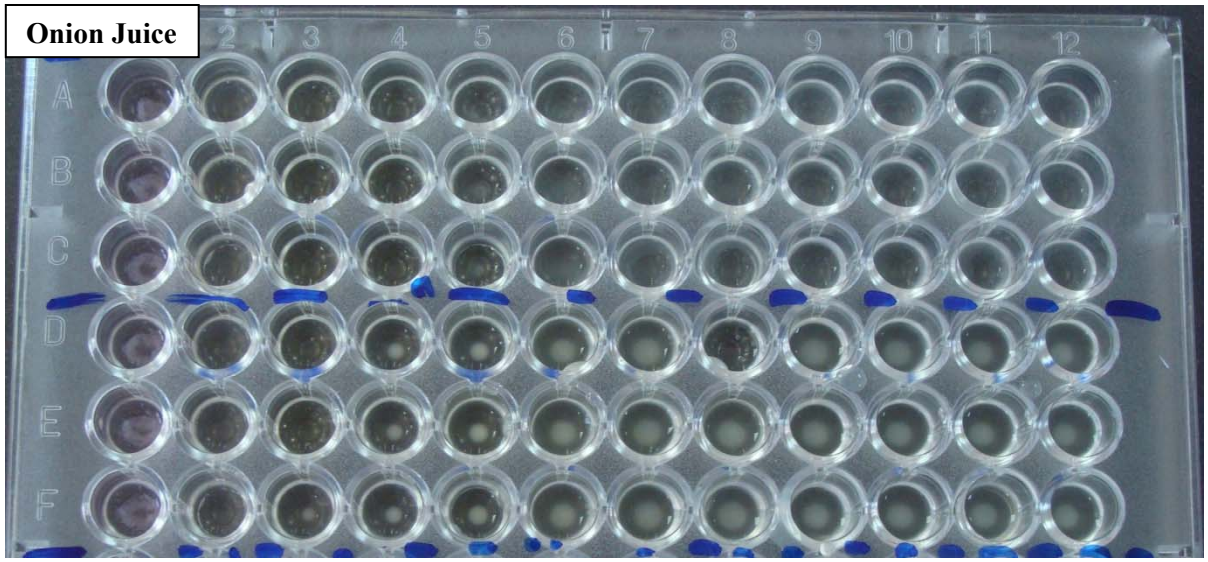


Figure 1: Microtiter plate with onion juice

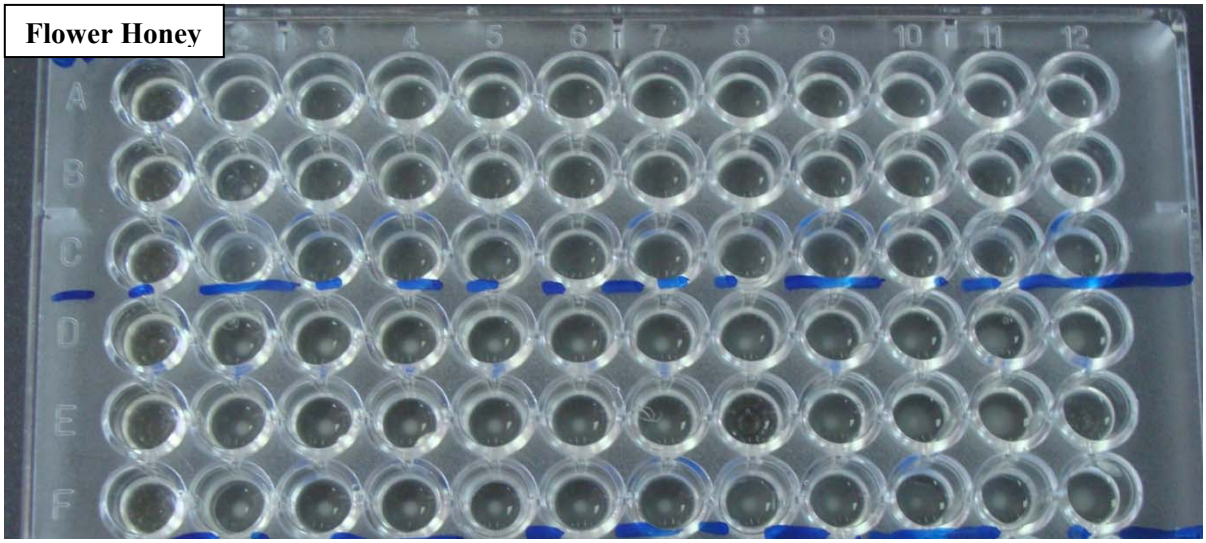


Figure 2: Microtiter plates with flower honey

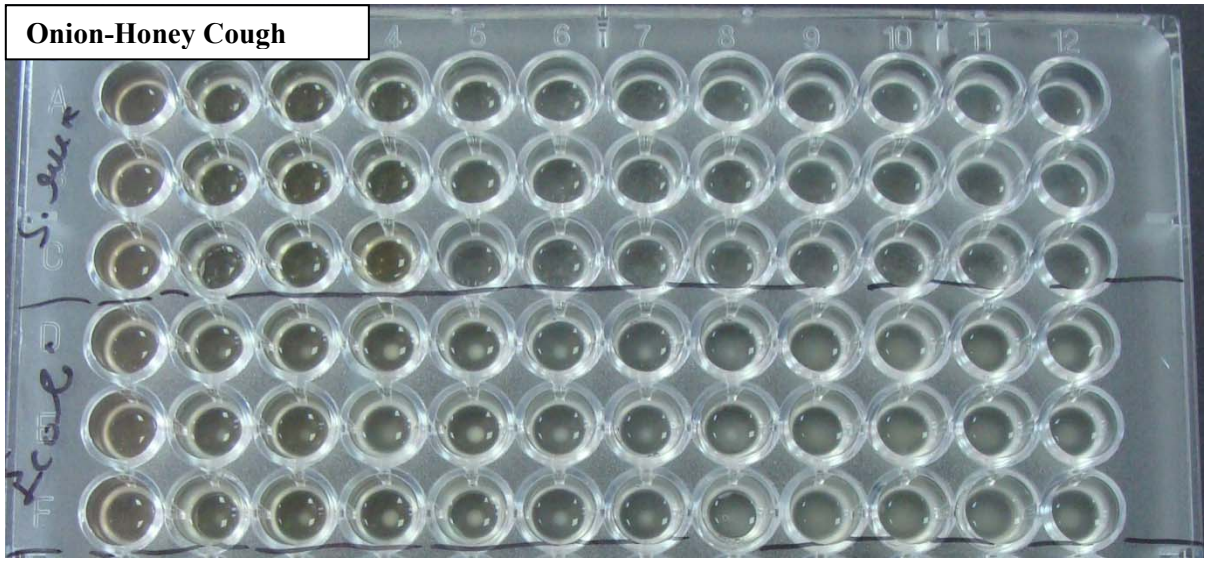


Figure 3: Microtiter plates with onion-honey cough cure

METHOD

Materials and Apparatus

1 bulb of red onion

Flower honey

Mueller Hinton II Broth

2 volumetric flasks of 100 mL

Ethanol

Sterile gauze

3 Falcon tubes

2 test tubes

Saline

Densitometer

Cotton swabs

6 microtiter plates (6x12)

Multi channel automatic pipettes of 100 µL and 10 µL

E. Coli ATCC 25922 in petri dish

S. aureus ATCC 25923 in petri dish

Incubator

Knife

1 glass beaker of 500 mL

Bunsen burner

3 petri dishes

Thermometer (±0.5 ºC)

Procedure

1. Onionskin was peeled with the help of a knife and the onion was sliced into eight pieces.
2. The onion pieces were placed in the beaker which was filled with 250 mL ethanol. It was closed with a thin layer of sterile gauze and it was left until the ethanol fully vaporized.
3. The blender was washed in dish washer to sterilize it and it was used to make onion paste. The resulting paste was squeezed in a piece of gauze to obtain onion juice. The falcon tube was filled with approximately 50 cc onion juice and it was stored in the fridge at about 4ºC for 24 hours.
4. The volumetric flask was filled with 15 cc flower honey and 15 cc Mueller Hinton II Broth. The volumetric flask was heated to 35 ºC slowly on Bunsen burner until honey and Mueller Hinton II Broth mixed to form 50% honey mixture.
5. 15 cc onion juice was poured into one of the petri dishes and 15 cc 50% flower honey mixture was put into another one. With the help of a volumetric flask, 15 cc honey and 15 cc onion juice was mixed & heated with Bunsen burner at 35 ºC to obtain homogeneous %50 honey-%50 onion juice mixture. The mixture obtained was then transferred to the third petri dish.

6. One microtiter plate was taken and the first cells of the six rows were filled with 200 µL 100% onion juice. All the other cells were filled with 100 µL Mueller Hinton II Broth.
7. By using the multi channel automatic pipettes, 100 µL 100% onion juice was taken back from the first cells of the six rows and it was transferred to the second cells of all the rows. With the small movements of the controller of the pipettes, onion juice- Mueller Hinton II Broth mixture was taken back into the pipettes and given back to the second cells twice. That helped the fully mixing of 100 µL 100% onion juice and 100 µL Mueller Hinton II Broth to form 200 µL 50% onion juice.
8. Similarly, 100 µL 50% onion juice was taken back from the second cells of the six rows and they are transferred to the third cells of the rows with the help of the multi channel automatic pipettes. With the small movements of the controller of the pipettes, onion juice- Mueller Hinton II Broth mixture was taken back into the pipettes and given back to the third cells twice. That helped the fully mixing of 100 µL 50% onion juice and 100 µL Mueller Hinton II Broth to form 200 µL 25% onion juice.
9. This dilution process was repeated until reaching the twelfth cell of the rows. There 100 µL Mueller Hinton II Broth was left alone to create a control group.
10. The other two microtiter plates were also filled with flower honey and onion-honey cough cure as prepared in Steps 6- 8.

	1	2	3	4	5	6	7	8	9	10	11	12
A	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.00%
B	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.00%
C	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.00%
D	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.00%
E	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.00%
F	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.00%

Figure 4: Distribution of concentrations for onion juice and onion-honey cough cure

	1	2	3	4	5	6	7	8	9	10	11	12
A	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.05%	0.00%
B	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.05%	0.00%
C	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.05%	0.00%
D	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.05%	0.00%
E	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.05%	0.00%
F	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.05%	0.00%

Figure 5: Distribution of concentrations for flower honey

11. Since the three rows of the microtiter plates would be used to inoculate E. coli and the other three rows would be used to inoculate S. aureus, we needed 2 more trials to reach 5 trials. Because of that, other three microtiter plates were filled with onion juice, flower

honey and onion-honey cough cure but that time, four rows of the microtiter plates were used (2 rows for E. coli, 2 rows for S. aureus)

12. Two test tubes were filled with saline. The first test tube was placed in the densitometer and E. coli was added from the petri dish into it with the help of a cotton swab until the densitometer shows 0.5 McFarland. 2 cc of saline-E. coli solution was transferred into 18 cc Mueller Hinton II Broth in a Falcon tube to obtain %10 E. coli solution. The same procedure was repeated to obtain 10% S. aureus solution.

13. Three rows of the microtiter plates that were prepared in Steps 6-10 were filled with 10 µL 10% S. aureus solution and the other three rows were filled with 10 µL 10% E. coli solution. Two rows of the microtiter plates that were prepared in Step 11 were filed with 10 µL 10% S. aureus solution and the other two rows were filled with 10 µL 10% E. coli solution.

14. After all of the six microtiter plates were filled with the active substances & bacteria, they were placed in the incubator which was adjusted to 37 ºC. Result of the experiment was taken after 24 hours.

RESULTS

Table 1. Reproduction of *S. aureus* and *E. coli* in flower honey of different concentrations after 24 hours at 37 ºC ± 0.1 ºC.

Growth medium used: Mueller Hinton II Broth

(+) sign represents reproduction, (-) sign represents no reproduction.

	FLOWER HONEY											
	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	0.05%	Pure Agar
S. aureus	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+
E. coli	-	+	+	+	+	+	+	+	+	+	+	+
	-	+	+	+	+	+	+	+	+	+	+	+
	-	+	+	+	+	+	+	+	+	+	+	+
	-	+	+	+	+	+	+	+	+	+	+	+
	-	+	+	+	+	+	+	+	+	+	+	+

Table 2. Reproduction of *S. aureus* and *E. coli* in onion juice of different concentrations after 24 hours at 37 ºC ± 0.1 ºC.

Growth medium used: Mueller Hinton II Broth

(+) sign represents reproduction, (-) sign represents no reproduction.

	ONION JUICE										
	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	Pure Agar
S. aureus	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
E. coli	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+
	-	-	+	+	+	+	+	+	+	+	+	+

Table 3. Reproduction of *S. aureus* and *E. coli* in onion-honey cough cure of different concentrations after 24 hours at 37 ºC ± 0.1 ºC.

Growth medium used: Mueller Hinton II Broth

(+) sign represents reproduction, (-) sign represents no reproduction.

	ONION-HONEY COUGH CURE											
	100.00%	50.00%	25.00%	12.50%	6.25%	3.13%	1.56%	0.78%	0.39%	0.19%	0.10%	Pure Agar
S. aureus	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
	-	-	-	-	+	+	+	+	+	+	+	+
E. coli	-	+	+	+	+	+	+	+	+	+	+	+
	-	+	+	+	+	+	+	+	+	+	+	+
	-	+	+	+	+	+	+	+	+	+	+	+
	-	+	+	+	+	+	+	+	+	+	+	+
	-	+	+	+	+	+	+	+	+	+	+	+

DATA ANALYSIS

Statistical Analysis: Chi-square test was applied with SPSS to compare the effect of active substances (flower honey, onion juice, and onion-honey cough cure) on *E. coli* and *S. aureus* to understand whether they can be considered to be same.

Statistical analysis needs to be numerical. So, each (+) sign on Table 1, Table 2 and Table 3 will be replaced by "1" and each (-) sign on Table 1, Table 2 and Table 3 will be replaced by "0".

Active Substance: Flower Honey

Ho: μ=0

There is no significant difference between the effect of flower honey on *S. aureus* and *E.coli*.

Bacteria * Trial Crosstabulation(a)

Count

		Trial		
		No reproduction	Reproduction	Total
Bacteria	S. aureus	2	9	11
	E. coli	1	10	11
Total		3	19	22

a. Active substance = Flower Honey

Table 4. Results of the reproduction of *E. coli* and *S. aureus* in flower honey medium.

Chi-Square Tests©

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.386	1	0.534		
Continuity Correction(a)	0.000	1	1.000		
Likelihood Ratio	0.392	1	0.531		
Fisher's Exact Test				1.000	0.500
Linear-by-Linear Association	0.368	1	0.544		
N of Valid Cases	22				

a. Computed only for a 2x2 table

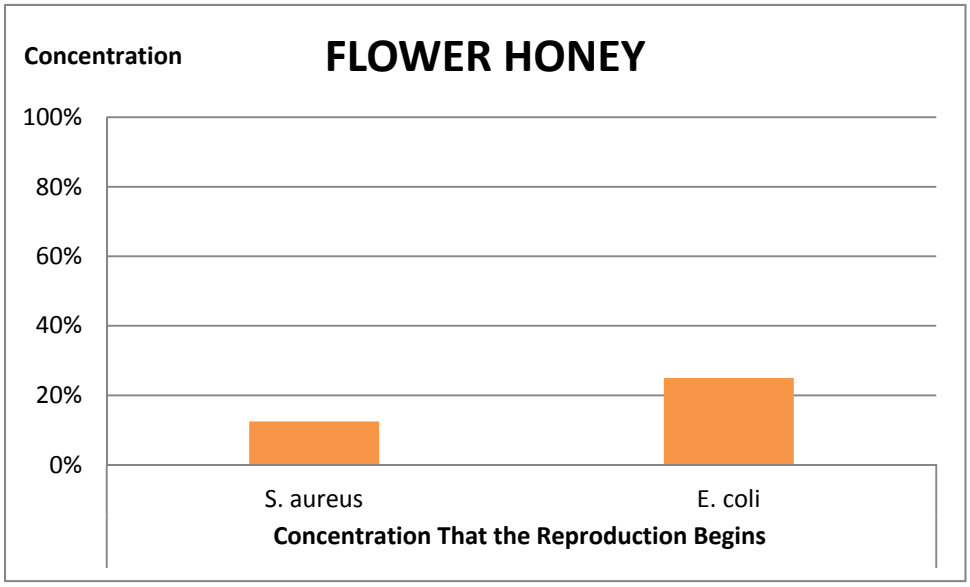
b. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 1,50.

c. Active_substance = Flower Honey

Table 5. Results of Chi-square test for flower honey medium.

p=0.534

Since p>0.05, the null hypothesis can be accepted. This means that there is no significant difference between the effect of flower honey on *S. aureus* and *E. coli*.



Graph 1. Concentration of the flower honey that the reproduction of *S. aureus* and *E. coli* can continue.

Active Substance: Onion Juice

Ho: μ=0

There is no significant difference between the effect of onion juice on *S. aureus* and *E. coli*.

Bacteria * Trial Crosstabulation(a)

Count

		Trial		
		No reproduction	Reproduction	Total
Bacteria	S. aureus	4	7	11
	E. coli	2	9	11
Total		6	16	22

a. Active_substance = Onion Juice

Table 6. Results of the reproduction of *E. coli* and *S. aureus* in onion juice medium.

Chi-Square Tests(c)

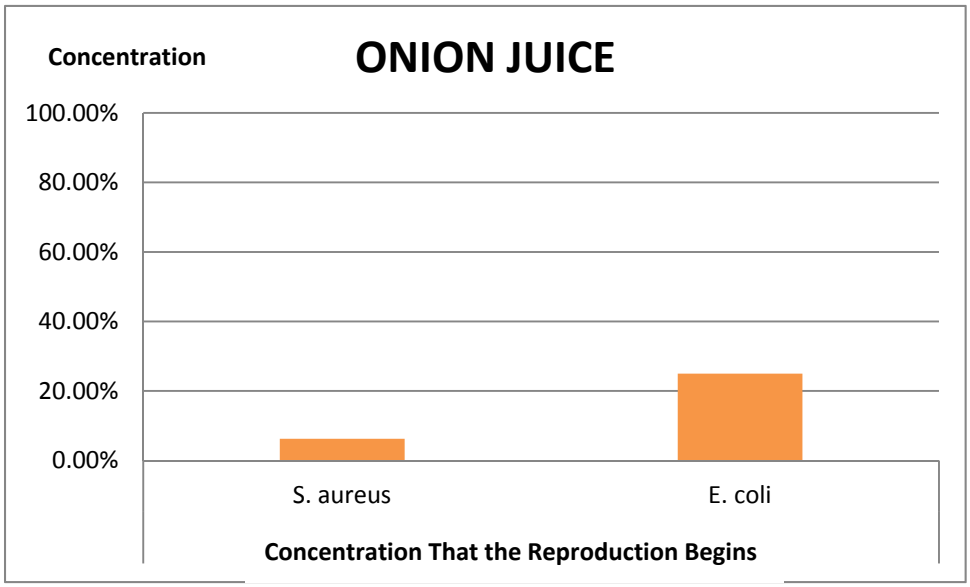
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.917	1	0.338		
Continuity Correction(a)	0.229	1	0.632		
Likelihood Ratio	0.930	1	0.335		
Fisher's Exact Test				0.635	0.318
Linear-by-Linear Association	0.875	1	0.350		
N of Valid Cases	22				

a. Computed only for a 2x2 table
b. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 3,00.
c. Active_substance = Onion Juice

Table 7. Results of Chi-square test for onion juice medium.

p=0.338

Since p>0.05, the null hypothesis can be accepted. This means that there is no significant difference between the effect of onion juice on *S. aureus* and *E. coli*.



Graph 2. Concentration of the onion juice that the reproduction of *S. aureus* and *E. coli* can continue.

Active Substance: Onion-Honey Cough Cure

Ho: μ=0

There is no significant difference between the effect of onion-honey cough cure on *S. aureus* and *E. coli*.

Bacteria * Trial Crosstabulation(a)

Count

		Trial		
		No reproduction	Reproduction	Total
Bacteria	S. aureus	4	7	11
	E. coli	1	10	11
Total		5	17	22

a. Active_substance = Onion-Honey Cough Cure

Table 8. Results of the reproduction of *E. coli* and *S. aureus* in onion-honey cough cure medium.

Chi-Square Tests(c)

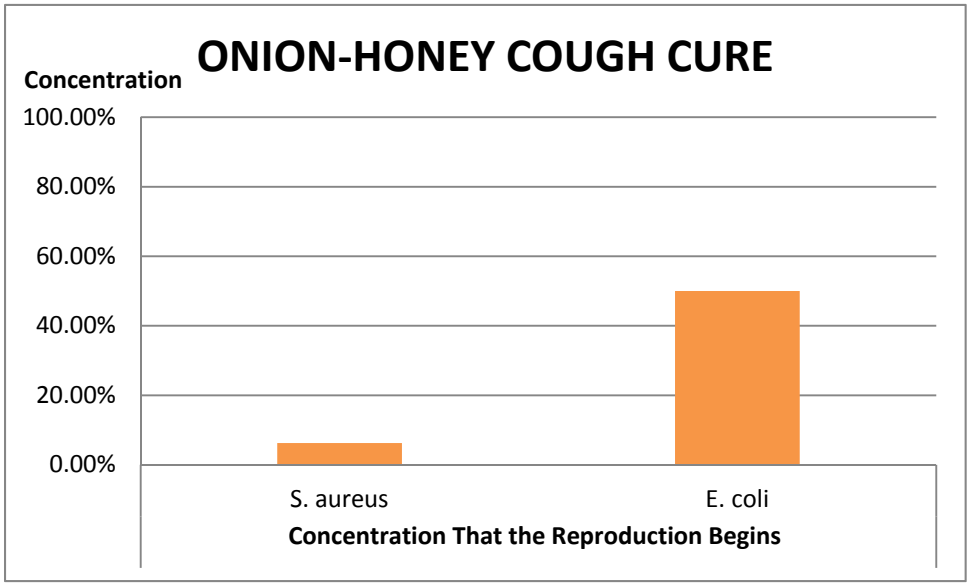
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.329	1	0.127		
Continuity Correction(a)	1.035	1	0.309		
Likelihood Ratio	2.460	1	0.117		
Fisher's Exact Test				0.311	0.155
Linear-by-Linear Association	2.224	1	0.136		
N of Valid Cases	22				

a. Computed only for a 2x2 table
b. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 2,50.
c. Active_substance = Onion-Honey Cough Cure

Table 9. Results of Chi-square test for onion-honey cough cure medium.

p=0.127

Since p>0.05, the null hypothesis can be accepted. This means that there is no significant difference between the effect of onion-honey cough cure on *S. aureus* and *E. coli*.



Graph 3. Concentration of the onion-honey cough cure that the reproduction of *S. aureus* and *E. coli* can continue.

Bacteria: S. aureus

Ho: μ=0

There is no significant difference between the effect of honey, onion juice and onion-honey cough cure on *S. aureus*.

Active_substance * Trial Crosstabulation(a)

Count

		Trial		
		No reproduction	Reproduction	Total
Active_substance	Flower Honey	2	9	11
	Onion Juice	4	7	11
	Onion-Honey Cough Cure	4	7	11
Total		10	23	33

a. Bacteria = S. aureus

Table 10. Results of the reproduction of *S. aureus* in flower honey, onion juice and onion-honey cough cure mediums.

Chi-Square Tests(b)

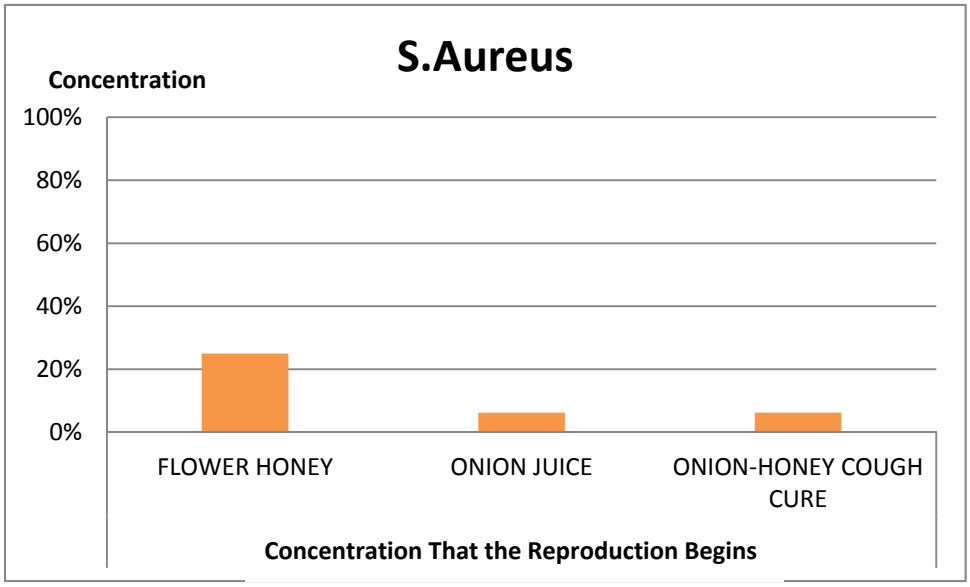
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.148	2	0.563
Likelihood Ratio	1.213	2	0.545
Linear-by-Linear Association	0.835	1	0.361
N of Valid Cases	33		

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 3,33.
b. Bacteria = S. aureus

Table 11. Results of Chi-square test for *S. aureus* in flower honey, onion juice and onion-honey cough cure mediums.

p=0.563

Since p>0.05, the null hypothesis can be accepted. This means that there is no significant difference between the effects of flower honey, onion juice and onion-honey cough cure on *S. aureus*.



Graph 4. Concentrations of flower honey, onion juice and onion-honey cough cure that *S. aureus* can continue reproduction.

Bacteria: E. coli

Ho: μ=0

There is no significant difference between the effects of honey, onion juice and onion-honey cough cure on *E. coli*.

Active_substance * Trial Crosstabulation(a)

Count

		Trial		
		No reproduction	Reproduction	Total
Active_substance	Flower Honey	1	10	11
	Onion Juice	2	9	11
	Onion-Honey Cough Cure	1	10	11
Total		4	29	33

a. Bacteria = E. coli

Table 12. Results of the reproduction of *E. coli* in flower honey, onion juice and onion-honey cough cure mediums.

Chi-Square Tests(b)

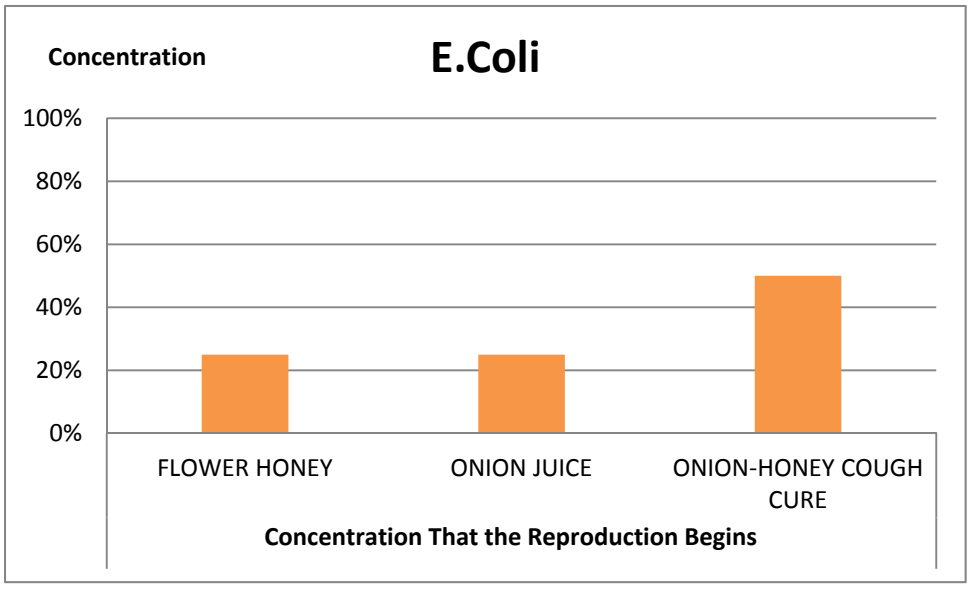
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	0.569	2	0.752
Likelihood Ratio	0.541	2	0.763
Linear-by-Linear Association	0.000	1	1.000
N of Valid Cases	33		

a. 3 cells (50,0%) have expected count less than 5. The minimum expected count is 1,33.
b. Bacteria = E. coli

Table 13. Results of Chi-square test for *E. coli* in flower honey, onion juice and onion-honey cough cure mediums.

p=0.752

Since p>0.05, the null hypothesis can be accepted. This means that there is no significant difference between the effects of flower honey, onion juice and onion-honey cough cure on *E. coli*.



Graph 5. Concentrations of flower honey, onion juice and onion-honey cough cure that *E. coli* can continue reproduction.

EVALUATION

My hypothesis was "The onion-honey cough cure will have a greater antibacterial effect on *S. aureus* and *E. coli* and the gram negative bacteria, E. coli, will be less affected from the antibacterial agents compared to the gram-positive bacteria, S. aureus." Results of the experiments showed that the hypothesis cannot be fully accepted.

In the microtiter plates with flower honey, reproduction of the *S. aureus* had started in the sample wells with 12.5% flower honey while the reproduction of the *E. coli* had started in the sample wells with 50% flower honey (See Table 1 and Graph 1). In the microtiter plates with onion juice, reproduction of *S. aureus* had started in the sample wells with 6.25% onion juice while the reproduction of *E. coli* had started in the sample wells with 25% onion juice (See Table 2 and Graph 2). Similarly, in the microtiter plates with onion-honey cough cure, reproduction of *S. aureus* had started in the sample wells with 6.25% onion-honey cough cure while the reproduction of *E. coli* had started in the sample wells with 50% onion-honey cough cure (See Table 3 and Graph 3).

This data shows us that flower honey and onion-honey cough cure have the same antibacterial effect on *E. coli* while the onion juice has a greater effect. On the other hand, antibacterial effect of onion juice and onion-honey cough cure is the same and stronger than the antibacterial effect of flower honey for *S. aureus*. Besides that, since the reproduction of *E. coli* had started in greater concentrations compared to *S. aureus*, it can be said that the susceptibility of *E. coli* towards the flower honey, onion juice and onion-honey cough cure was always lower compared to *S. aureus*. Since onion-honey cough cure has the same antibacterial property with the flower honey for *E. coli* and it has the same antibacterial property with onion juice for *S. aureus*, it cannot be accepted that onion-honey cough cure has a greater antibacterial effect compared to onion juice and flower honey. On the other hand, it is true that *E. coli*, a gram-negative bacterium is more susceptible to the antibacterial agents used in the experiment compared to *S. aureus*. The reason for that might be the special structure of the cell wall of gram negative bacteria as explained in Hypothesis part.

Although the graphs clearly show that there is a difference between the susceptibility of the *S. aureus* and *E. coli* in the mediums with honey, onion juice and onion-honey cough cure, Chi-square test shows the exact opposite. Table 5 shows that there is no significant difference between the effect of flower honey on *S. aureus* and *E. coli* since p value equals to 0.534. It is also possible to say that there is no significant difference between the effect of

onion juice and onion-honey cough on *S. aureus* and *E. coli* since p values are both bigger than 0.05. This means that the difference between the effectiveness of the antibacterial agents could not be proven statistically. These unexpected results are due to unbalanced distribution of the data. Since the number of cells that reproduction was not observed is low compared to the number of cells that reproduction was observed, results were not 100% safe. This situation can also be understood from the explanation given under the Table 5, 7,9,11 and 13. It says the cells have expected count less than 5.

The main problem with the experiment is a limitation that the method I had chosen before caused. The flower honey was so dense to transfer with the pipette so I had to start experiment with 50% honey-Mueller Hinton II Broth mixture. This is not a problem while considering the effect of flower honey on *S. aureus* and *E. coli* but since the maximum concentration of the honey mixture is 50%, I had to use 50% onion juice to produce 1-1 onion-honey cough cure. If the onion-honey cough cure could have been produced with 100% onion juice and 100% flower honey, onion-honey cough cure could have had greater antibacterial effects on *S. aureus* and *E. coli* and the hypothesis could be accepted.

To overcome the problem with the concentration of honey and statistics, another antimicrobial susceptibility test could have been used like the disc diffusion test as discussed in the Method Development part. This method could have changed the results in two ways: First of all, greater concentration of honey could have been used. Secondly, since the susceptibility of the bacteria is measured according to the zone length, numerical results would be obtained instead of 1's (Reproduction occurred.) and 0's (Reproduction did not occur.), and the Chi-square test could have given the expected results.

Another problem was the usage conditions of the incubator. I was not the only person who used it during the experiment. When I left my microtiter plates in the incubator for 24 hours, other people also used it to place their experiments. As indicated in the Method Development part, an unknown kind of bacterium had produced in one of the microtiter plates and spread to others although the microtiter plates are closed with a top. Because of that, I had to repeat my experiment.

CONCLUSION

The results of the experiment showed that flower honey, onion juice and onion-honey cough cure have antibacterial effects on both chosen Gram-positive and Gram-negative bacteria, *S. aureus* and *E. coli* although the antibacterial effect of the onion-honey cough cure was not as strong as expected. In that way, my experiment shows difference from the experiment of Al Masaudi and Al Bureikan because their research showed that the combination of onion-honey has stronger growth reduction and enhancing the killing activity on microorganisms than onion alone or honey alone. On the other hand, their research was also showing that *S. aureus* was more sensitive to honey compared to *E. coli*.[9] The difference between the results might be due to the concentration of onion-honey cough cure as discussed in Evaluation part or it can also be about the type of the honey or onion used.

During my researches, I saw that garlic and ginger have similar antibacterial effects with onion. Because of that, in another experiment, the antibacterial property of garlic-honey mixture or honey-ginger mixture can be investigated and compared to each other t see which one has greater antibacterial effects.

The results of the experiment brought my mind an important question: Is it possible to say that the active substances used in the experiment would have similar antibacterial effects on the people who use it? The strain of the S. aureus and E. coli used in the experiment was standard and a very little amount of them were used. When it comes to the real life, same kinds of bacteria may have different resistance qualities towards different substances so it is not possible to say that onion juice, flower honey and onion-honey cough cure will always have the same effect on the given bacteria.

Although onion juice was more effective on *E. coli* and *S. aureus* had affected equally from the onion juice and onion-honey cough cure, it is a better choice to drink onion-honey cough cure since they create a perfect medium to enhance bacterial growth together: honey has many antibacterial properties due to its osmotic pressure, pH and H_2O_2 it contains where onion contains organosulphur compounds that have antibacterial effects as indicated in the Introduction part. Since it is not possible to know which bacterium has made you ill, it is the safest option to have onion-honey cough cure.