TED ANKARA COLLEGE FOUNDATION PRIVATE HIGH SCHOOL

BIOLOGY

EXTENDED ESSAY

Comparing the antibacterial effect of *Chichorium intybus* and *Urtica dioica* on *Staphylococcus aureus* as gram positive bacterium and *Escherichia coli* as gram negative bacterium

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Word Count: 3630

Abstract

People use antibacterial medicines to defend themselves against bacterial diseases. However as these synthetic medicines may have harmful and unexpected effects on human health, people prefer using herbal medicines instead of synthetic medicines. Depending on this fact I defined my research question as; "How does the antibacterial effect of *Chichorium intybus* and *Urtica dioica* change on *Staphylococcus aureus* as gram positive bacterium and *Escherichia coli* as gram negative bacterium?". There are lots of studies about antibacterial effect, but I did not run into studies about antibacterial effects of different plants on gram positive and gram negative bacteria. So, the aim of this study is comparing the antibacterial effect of two different plants (*Chichorium intybus* and *Urtica dioica*) on gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacterium and it was hypothesized that, "*Chichorium intybus* and *Urtica dioica* both have significant antibacterial effect on both gram positive and gram negative types of bacteria."

In this experiment, antibacterial effect of plants (*Chichorium intybus* and *Urtica dioica*) on gram positive and gram negative bacteria (*Staphylococcus aureus* and *Escherichia coli*) is measured by preparing Mueller - Hinton Agar and Whatman No. 1 filter paper which is absorbed with plant extracts and their inhibition zones are measured. For *Staphylococcus aureus*, *Chichorium intybus* gives 11.0mm average inhibition zone radius and *Urtica dioica* gives 9.1mm average inhibition zone radius. For *Escherichia coli*, *Chichorium intybus* gives 9.1mm average inhibition zone radius and *Urtica dioica* gives 9.2mm average inhibition zone radius.

For evaluating these results, t-test is made and p value of effect on *Escherichia coli* is bigger than 0.05 so there are no significant differences between antibacterial effects of these two plants, p value of effect on *Staphylococcus aureus* is smaller than 0.05 so the null hypothesis is rejected and hypothesis is accepted according to this study.

Word Count: 300

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I. INTRODUCTION / BACKGROUND

The first time I was confronted with this topic was when I realized my mother's interest in the effects of some herbs. She loves examining the effects of them on human health. So, her interest increased my curiosity about this subject. I decided to turn my mother's non-sciencely efforts to something scientific. While I was talking with my mother about my research, she advised me to study about chicory and nettle because she had experienced that they have some positive effects on health. So, I started to make a background research about these plants.

Chicory (*Chichorium Intybus*) is a plant from Asteraceae family which is the largest family of vascular plants. It usually has bright blue flowers. Its stem is generally 30-100cm tall. Its leaves are lanceolate and stalked. The flower part is 2-4 cm wide.[1] In daily life it has some practical benefits against some diseases. In my country, people use it for many purposes such as skin beauty and its help for diabetes patients. It has an important effect on distribution of blood to all body cells effectively. Chicory leaves are eaten raw as salad leaves after some cultivation. Chicory is a very rich source of vitamin A, and it is very good for eyes. [2]

Nettle is a plant that even its leaves, seeds and roots have effective character. In ancient times, it also had a great reputation. Albrecht Dürer painted flying of an angel to God with a nettle in its hand.[3] Most of the scientists say that if nettle hasn't been protected by its burning property (with histamine and acetylcholine on its hairs), the plant would have been extinguished already.

Large stinging nettle (Urtica diocia L.) is perennial and herbaceous plant and sometimes its height can be over 1 meter. Leaves are dark green, stalked, toothed edges and hairy with burning property. Nettle's leaves contain flavones, magnesium, vitamin c, iron, mineral salts, plant acids, betasitosterin, sterylglucosid and lignans. Seeds contain, mucilages, proteins, fixed oil, is carotinoid and clorophyll. And roots contain tannin, sterolen, sterylglucosid and lignans.[4] In addition to magnesium, studies

[1] http://en.wikipedia.org/wiki/Chicory
[2] http://www.online-vitamins-guide.com/herbs/chicory.htm
[3] http://www.bitkisel-tedavi.com/isirgan.htm
[4] http://www.bitkisel-tedavi.com/isirgan.htm

showed that nettle tea carries anti-bacterial properties. Mouth rinse and tooth paste containing nettle fluids, mostly reduces the formation of plaque on teeth.[5]

"Historically, stinging nettle has been used to treat muscle pain, joint pain, eczema, arthritis, gout, and anemia. It is also now used to treat urinary problems associated with benign prostatic hyperplasia, urinary tract infections, hay fever, tendinitis, insect bites and more. Studies have shown that the extract of the stinging nettle leaf suppresses cytokines associated with inflammatory joint disease. Aside from pain-relieving properties, stinging nettle has also been shown to have anti-bacterial and anti-ulcer effects." [6]

I choose these plants because I could find them easily and their antibacterial effects can be observed clearly. These plants have many different properties as mentioned above. They have lots of useful properties which I can use in the experiment and many of their properties are known by people, so these plants are perfect for this experiment.

I want to use two different types of bacteria because I want to observe different effects of antibacterial plants on gram positive and gram negative bacteria types. For this experiment I want to increase the options which can give me different results so one reason that I use two different types of bacteria can be count as this.

Two types of bacterium was used in this study other types of bacterium can give different reactions to these plant extracts.

Staphylococcus aureus is a gram positive bacterium. *"It is frequently found as part of the normal skin flora on the skin and nasal passages. It is estimated that 20% of the human population are long-term carriers of S. aureus. S. aureus is the most common species of staphylococci to cause Staph infections. The reasons S. aureus is a successful pathogen are a combination host and bacterial immuno-evasive strategies"*[7]

[5] http://www.medikal-hastaliklar.com/diseti-iltihaplanmasina-bitkisel-cozum.html
[6] http://osteoarthritis.about.com/od/alternativetreatments/a/stinging_nettle.htm
[7] http://en.wikipedia.org/wiki/Staphylococcus_aureus

This bacterium is chosen for this study because it has a pathogen behavior and causes lots of diseases. It's too easy to obtain this bacterium. *Staphylococcus aureus* fairly show the characteristic properties of gram positive bacteria about cell membranes.

Escherichia coli are gram negative bacterium. *"E-coli are rod shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most E. coli strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator to test environmental samples for fecal contamination. The bacterium can also be grown easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. E. coli is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology"*[8]

This bacterium is chosen in this study because E-coli is pathogen and it reproduce quickly. We know all the properties of this bacterium and we know it can cause lots of diseases. It's too easy to obtain this bacterium too. *Escherichia coli* fairly show the characteristic properties of gram negative bacteria about cell membranes.

For this extended essay experiment, as I showed the properties above, *Chichorium intybus* and *Urtica dioica* are the best plants for investigating antibacterial effects. I want to investigate both the effects of gram positive and gram negative bacteria so *Staphylococcus aureus* and *Escherichia coli* are the best bacterium to choose because they show the characteristic properties of their categories. So, these bring me to the research question.

[8] http://en.wikipedia.org/wiki/Escherichia_coli

Research question: How does the antibacterial effect of *Chichorium intybus* and *Urtica dioica* change on *Staphylococcus aureus* as gram positive bacterium and *Escherichia coli* as gram negative bacterium?

II. HYPOTHESIS

Chichorium intybus and *Urtica dioica* are two herbs which are known for their many different and positive effects on human health. As they have lots of effects their effects are generally different from each other. *Urtica dioica* have lots of beneficial properties for people. For example, it defends wounds against microbes. Also, *Urtica dioica* is a diuretic substance. It also quenches the rheumatic pains. It is said that *Urtica dioica* can be used in treating cancer but there are no proved scientific facts.[9] C*hichorium intybus* have also beneficial properties for people. It purges the blood and annihilates toxins from liver.[10] It also controls the level of sugar. It fights with worms and parasites which are found in intestine.[11] So I decided to check their antibacterial effects to learn more about their effects on health and also to compare them. To broaden my research I used two different types of bacteria one is belonging to gram positive type and the other to gram negative type. I expect that they both have antibacterial effect on both types of bacteria.

"In this study, water extract of nettle ***(Urtica dioica*** *L.) (WEN) was studied for antioxidant, antimicrobial, antiulcer and analgesic properties…WEN exhibited antimicrobial activity against all tested microorganisms. Of the species used, Staphylococcus aureus is one of the most common Gram-positive bacteria causing food poisoning. Its source is not the food itself, but the humans who contaminate food after if has been processed (Rauha et al., 2000). Interestingly WEN showed antibacterial activity against this bacterium."*[12]

My hypothesis is: "*Chichorium intybus* and *Urtica dioica* both have significant antibacterial effect on both gram positive and gram negative types of bacteria."

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[9] Saraç, Dr. Ender. Doğanın Şifalı Eli. İstanbul: Doğan Kitap, 2005.
[10] http://www.liveandfeel.com/medicinalplants/chicory.html
[11] http://www.natural-homeremedies.org/blog/health-benefits-of-chicory/
[12] **Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.)**
By İlhami Gülçin, Ö. İrfan Küfrevioğlu, Münir Oktay, Mehmet Emin Büyükokuroğlu
URL: http://esa.ipb.pt/pdf/RefPlants_20.pdf

III. METHOD DEVELOPMENT AND PLANNING

The first aim of this extended essay was; investigating the antibacterial effect of leaf and root parts of *Urtica dioica.* Experiment is done and no zones are formed. So, I change the aim. The reason that no zones are formed can be the place and the way which the *Urtica dioica* collected. There can be some differences between same plants which belong to different places. The way of collecting plants can also cause differences. So, I want to change my research question and made the experiment with two different plants and two different types of bacteria.

In this experiment 2 types of plants and 2 types of bacteria are used. *Chichorium intybus* and *Urtica dioica* are the plants that were used. These plants were dried in room temperature and shredded. The parts of plants which will be investigated for their antibacterial properties are taken and extracted at 80°C with %80 ethanol for 6 hours in soxhlet.

Extracts of ethanol volatilize in low pressure by filtering by Whatman No. 1 filter papers. (<40°C) The dry plant extracts is dissolved by dimethylsulfoxide and sterilized with the help of membrane filtration (0.45µm). Whatman No. 1 filter papers are used because they can easily left the solution which absorbed by paper by the help of diffusion so extract can easily go toward agar.

For investigating the antibacterial effects of plant extracts disc diffusion test is done and Mueller - Hinton Agar is used for it. **(see Appendix I.)** Mueller - Hinton Agar is solid and is suitable for diffusion. Another name for this property is being gelose. Its composition provides best conditions for developing. So development of bacterium is blocked by only the extract which experimenter put in it.

Antibacterial effect of extracts by qualitative observation;

Disc diffusion method is used for observing bacterium's sensibility to plant extracts. Disc diffusion method is appropriate for using in Mueller - Hinton Agar because the extracts which are absorbed by filter papers can easily diffuse and spread in the Mueller - Hinton Agar.

Papers, which are used for preparing discs, are called as Whatman No. 1 and they were cut with 6mm radius. Whatman No. 1 filter papers provide an easy way for extract to pass to Agar by diffusion.

30µl extract absorbed to discs and discs are dried at 30°C. We choose 30µl, because it is the optimum amount that filter papers can absorb without staying moist. We want the filter paper neither too moist, nor too dry.

As bacterium type; *Staphylococcus aureus* used as gram positive bacterium and *Escherichia coli* is used as gram negative bacterium. These bacteria are reproduced in the microbiology lab for me. Bacterium is set as 0.5 McFarland standards. **(see Appendix II.)** 0.5 McFarland is the optimum value in literature value which is found by lots of experiments.

Discs which are absorbed with extracts are placed on bacterium suspensions which are spread on agars with certain distances.

Discs which are absorbed with DMSO (Dimethylsulfoxide) are used for negative control and discs which are absorbed with gentamicin are used for positive control. Since DMSO doesn't have antibacterial property and has solvent property, DMSO is used as a solvent in this experiment.

Bacterium is left in incubation for 24 hours and 37°C, under aerobic conditions. Incubation is made in incubator. 37°C and 24 hours are best reproducing conditions for bacterium. Bacterium population reaches its maximum growth rate at those conditions. Incubation temperature is kept constant by the incubator.

After that time period, inhibition zones are observed as if they arise or not. For the antibacterial activity inhibition zones' radius (mm) is taken as basis. Inhibition zones with radius 8mm and more than 8mm inhibition levels evaluated. Because zones under 8mm don't have enough antibacterial effect to be evaluated.

Antibacterial effect of extracts *by* quantitative observation;

Plant extracts are diluted and samples are prepared in 4.0-0.015 mg/ml concentrations. Petri dishes which are prepared with filter papers which absorb these different concentrations of plant extracts are put in incubation. After this procedure, Petri dishes which formed 8mm zone are accepted as minimum incubation value which is used in the experiment. In disc diffusion method, for quantitative

measurement of antibacterial effects of plant extracts which have inhibition zones of 8mm and more than 8mm, broth macro dilution method is used and minimum inhibitor concentrations are determined. I did 20 trials for each bacteria type (*Staphylococcus aureus* and *Escherichia coli*) for both two plants (*Chichorium intybus* and *Urtica dioica*). I made this extended essay experiment in Gazi University Microbiology Laboratories with the help of Prof. Dr. Zeki Aytaç, Research Assistant Burcu Meryem Kavukoğlu and Research Assistant Feyza Öke. They helped me while I was doing the experiment about the method and they supported me with a rich supply of material.

Materials used in the experiment:

- *Urtica dioica* plant from Istanbul – Turkey
- *Chichorium intybus* from Istanbul – Turkey
- Pure strain non-antibiotic resistant *Staphylococcus aureus*
- Pure strain non-antibiotic resistant *Escherichia coli*
- For 40 Petri dish – Mueller - Hinton Agar
- For 40 Petri dish – Standard size Whatman No. 1 filter paper
- 40 x Petri dishes
- Soxhlet (for produce extracts)
- Incubator
- Millimetric ruler (±0.5mm)

IV. METHOD

1. Preparing of materials and cultures

a. Preparing of agar: Mueller - Hinton Agar is prepared and put into Petri dishes which sterilized in otoclave, for becoming solid.

b. Contamination test of agars: Agars which do not contaminate after putting them into incubator at 37°C for 24 hours, are used for the experiment.

2. Preparing the extracts

a. Plants parts are dried at room temperature in dark medium.

b. Extracts are prepared in soxhlet, volatilizing of ethanol and drying of extract. Preparing extracts for absorption by Whatman No. 1 filter papers by solve extracts in DMSO.

3. Preparing and applying of experiment:

Bacterium is spread with spread technique **(see Appendix III.)** and Whatman No. 1 filter papers which absorbed different concentrations of extract are put on Petri dishes. These Petri dishes are put in incubator at 37°C for 24 hours. After that period Petri dishes which have the 8mm or more than 8mm zones are evaluated.

4. Evaluation:

Collecting qualitative and quantitative data and evaluated by looking their zone diameters.

V. RESULTS

Table 1: The table below represents the antibacterial effects of *Chichorium intybus* and *Urtica dioica* on different types of bacteria (gram positive and gram negative) by the diameters of inhibition zones.

Type of Plant	Trials	Inhibition Zone on E-coli (±0.5mm)	Inhibition Zone on Staphylococcus aureus (±0.5mm)	Incubation Temperature (±0.5°C)	Place where Plant is collected	Plant Parts Used	Extract Amount (±0.1µl)
Chichorium intybus	1	10.1	11.2	37.0	Istanbul	Root	30.0
	2	11.2	11.0	37.0	Istanbul	Root	30.0
	3	8.0	9.8	37.0	Istanbul	Root	30.0
	4	9.1	12.0	37.0	Istanbul	Root	30.0
	5	8.7	12.1	37.0	Istanbul	Root	30.0
	6	9.2	11.7	37.0	Istanbul	Root	30.0
	7	8.9	10.4	37.0	Istanbul	Root	30.0
	8	8.1	10.0	37.0	Istanbul	Root	30.0
	9	9.9	10.1	37.0	Istanbul	Root	30.0
	10	8.0	11.2	37.0	Istanbul	Root	30.0
Urtica dioica	1	7.9	8.2	37.0	Istanbul	Leaf and top part with seed	30.0
	2	7.2	9.0	37.0	Istanbul	Leaf and top part with seed	30.0
	3	10.1	11.1	37.0	Istanbul	Leaf and top part with seed	30.0
	4	11.3	7.9	37.0	Istanbul	Leaf and top part with seed	30.0
	5	8.8	7.8	37.0	Istanbul	Leaf and top part with seed	30.0
	6	10.1	9.2	37.0	Istanbul	Leaf and top part with seed	30.0
	7	9.8	8.1	37.0	Istanbul	Leaf and top part with seed	30.0
	8	9.3	9.9	37.0	Istanbul	Leaf and top part with seed	30.0
	9	8.7	10.1	37.0	Istanbul	Leaf and top part with seed	30.0
	10	8.5	9.6	37.0	Istanbul	Leaf and top part with seed	30.0

VI. DATA ANALYSIS

Table 2: The table below represents the Statistical analysis of antibacterial effects of *Chichorium intybus* and *Urtica dioica* on different types of bacteria (gram positive and gram negative) by means of the diameters of inhibition zones.

- Inhibition Zone on *E-coli*
  (mm ±0.5)

	Chichorium intybus	*Urtica dioica*
Mean	9,1	9,2
Standard Error	0,3	0,4
Median	9,0	9,1
Mode	8,0	10,1
Standard Deviation	1,0	1,2
Sample Variance	1,1	1,4
Kurtosis	0,2	-0,1
Skewness	0,8	0,1
Range	3,2	4,1
Minimum	8,0	7,2
Maximum	11,2	11,3
Sum	91,2	91,7
Count	10,0	10,0
Confidence Level(95,0%)	0,7	0,9

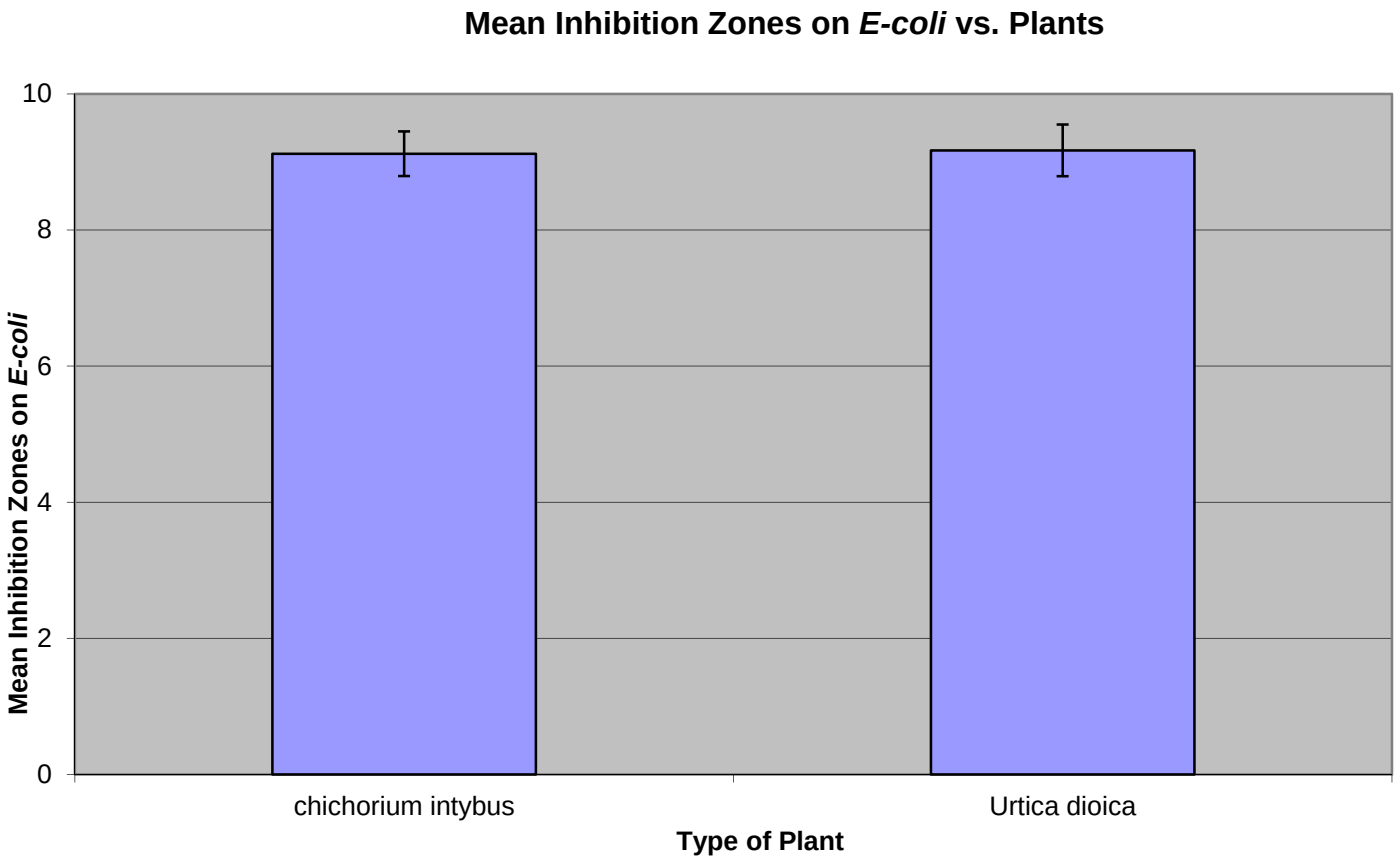
- Inhibition Zone on *Staphylococcus aureus*
  (mm ±0.5)

	Chichorium intybus	*Urtica dioica*
Mean	11,0	9,1
Standard Error	0,3	0,3
Median	11,1	9,1
Mode	11,2	--
Standard Deviation	0,8	1,1
Sample Variance	0,7	1,2
Kurtosis	-1,5	-0,7
Skewness	0,0	0,5
Range	2,3	3,3
Minimum	9,8	7,8
Maximum	12,1	11,1
Sum	109,5	90,9
Count	10,0	10,0
Confidence Level(95,0%)	0,6	0,8

For *E-coli*;

t-Test: Two-Sample Assuming Equal Variances

	chichorium intybus	*Urtica dioica*
Mean	9,12	9,17
Variance	1,075111111	1,442333333
Observations	10	10
Pooled Variance	1,258722222	
Hypothesized Mean Difference	0	
df	18	
t Stat	-0,099652926	
P(T<=t) one-tail	0,460860682	
t Critical one-tail	1,734063592	
P(T<=t) two-tail	**0,921721363**	
t Critical two-tail	2,100922037	

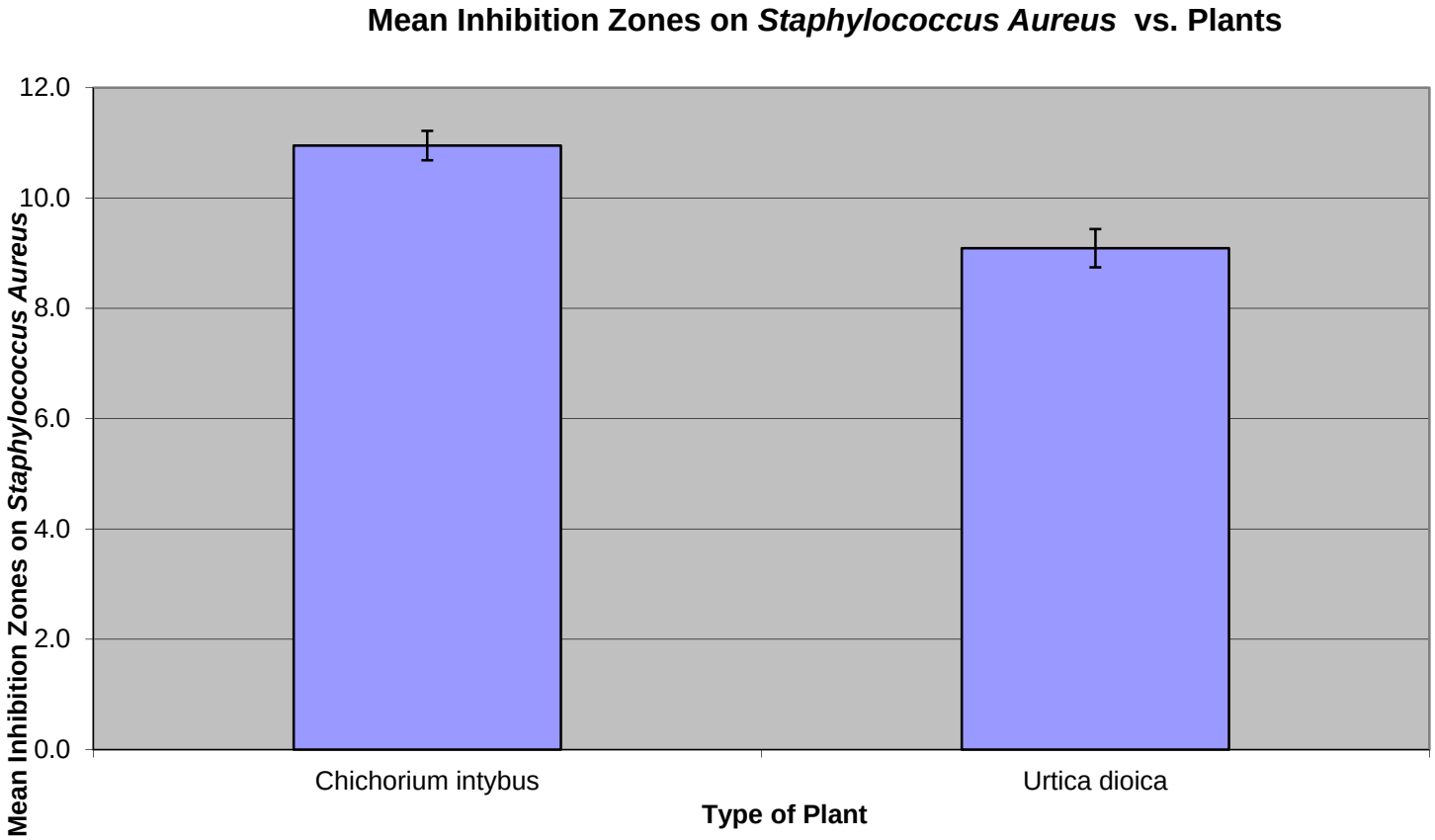


Graph 1: The comparison of the inhibition zones of plants on *E-coli*. Error bars show standard error for each group.

For *Staphylococcus aureus*;

t-Test: Two-Sample Assuming Unequal Variances

	chichorium intybus	*Urtica dioica*
Mean	10,95	9,09
Variance	0,707222222	1,205444444
Observations	10	10
Hypothesized Mean Difference	0	
df	17	
t Stat	4,252979601	
P(T<=t) one-tail	0,000268332	
t Critical one-tail	1,739606716	
P(T<=t) two-tail	**0,000536664**	
t Critical two-tail	2,109815559	



Graph 2: The comparison of the inhibition zones of plants on *Staphylococcus aureus.* Error bars show standard error for each group.

VII. EVALUATION

The aim of this study is to find the different antibacterial reactions of two different plants on two different types of bacterium. It was hypothesized that *Chichorium intybus* and *Urtica dioica* both have significant antibacterial effect on both gram positive and gram negative types of bacteria. Both plants showed antibacterial effect on both bacterium types. *Chichorium intybus* reacted to *Staphylococcus aureus* and a zone with mean 11.0 diameters is formed. *Urtica dioica* reacted to *Staphylococcus aureus* too and a zone with mean 9.1 mm diameter is formed. This shows us *Chichorium intybus* have more antibacterial effect than *Urtica dioica* on gram positive bacterium *Staphylococcus aureus.* The literature value which I have found in another research; inhibition zone of *Urtica dioica* on *Staphylococcus aureus* is about >15mm diameter. The research says that *Urtica dioica* gives "*a very good activity (>15mm)"* to *Staphylococcus aureus*. According to that research, in my experiment, *Urtica dioica* gives normal activity to *Staphylococcus aureus.* So my experiment's results are related to literature value. ([13] Table 2 in research, R. Singh, S.A. Dar and P. Sharma, 2012. Antibacterial Activity and Toxicological Evaluation of Semi Purified Hexane Extract of *Urtica dioica* Leaves. *Research Journal of Medicinal Plant, 6: 123-135.)* Another literature value is for *Chichorium intybus* in another research. In that research, *Chichorium intybus* gives antibacterial reaction to *Staphylococcus aureus* as a diameter of 9.8mm in hexane solution extract. According to that research, in my experiment, the inhibition diameter of *Chichorium intybus* on *Staphylococcus aureus* is 11.0mm, so results of my experiment related to literature value. ([14] Table 2 in http://idosi.org/abr/1(1-2)/2.pdf)

However, two plants react nearly equal to the gram negative bacterium *Escherichia coli*. *Chichorium intybus* reacted to *Escherichia coli* and a zone with mean 9.1mm diameter is formed and *Urtica dioica* reacted to *Escherichia coli* and a zone with mean 9.2 mm diameter is formed. These zone diameters

[13] R. Singh, S.A. Dar and P. Sharma, 2012. Antibacterial Activity and Toxicological Evaluation of Semi Purified Hexane Extract of *Urtica dioica* Leaves. *Research Journal of Medicinal Plant, 6: 123-135.*
DOI: 10.3923/rjmp.2012.123.135
URL: http://scialert.net/abstract/?doi=rjmp.2012.123.135

[14] http://idosi.org/abr/1(1-2)/2.pdf

are nearly equal and this shows us there are no significant differences between two plants' antibacterial effects on *Escherichia coli*. In the research which footnoted above as 10' , there is a literature value about antibacterial effect of *Urtica dioica* on gram negative bacteria *Pseudomonas aeruginosa*. The research says, *Urtica dioica* gives "*good activity(>10mm* - ≤15mm)*"* to *Pseudomonas aeruginosa.* According to that research, in my experiment, *Urtica dioica* gives 9.2mm inhibition zone, so results of my experiment are nearly related to literature value. ([15] Table 2 in research, R. Singh, S.A. Dar and P. Sharma, 2012. Antibacterial Activity and Toxicological Evaluation of Semi Purified Hexane Extract of *Urtica dioica* Leaves. *Research Journal of Medicinal Plant, 6: 123-135.)* Another literature value is for *Chichorium intybus* in another research. In that research, (which mentioned as footnote above as 11') *Chichorium intybus* gives antibacterial reaction to *Escherichia coli* as a diameter of 9.6mm in hexane solution extract. According to that research, in my experiment, the inhibition diameter of *Chichorium intybus* on *Escherichia coli* is 9.1mm, so results of my experiment related to literature value. ([16] Table 2 in http://idosi.org/abr/1(1-2)/2.pdf)

My null-hypothesis was there are no significant difference in antibacterial properties of different plants on gram positive and gram negative bacterium in terms of the diameter of their inhibition zones in Petri dishes.

For *Escherichia coli* since two – tail p value is 0,921721363 > 0.05; there are no significant differences between antibacterial effects of these two plants. Although they both have significant antibacterial effect to bacteria. We can see it in the data analysis that mean results are nearly the same. So we can easily say that on chosen gram negative bacteria, *Chichorium intybus* and *Urtica dioica* do not have different values of antibacterial effect. But for *Staphylococcus aureus*, two – tail p value is 0,000536664 <0.05; my null hypothesis is rejected according to this study.

[15] R. Singh, S.A. Dar and P. Sharma, 2012. Antibacterial Activity and Toxicological Evaluation of Semi Purified Hexane Extract of *Urtica dioica* Leaves. *Research Journal of Medicinal Plant, 6: 123-135.*
DOI: 10.3923/rjmp.2012.123.135
URL: http://scialert.net/abstract/?doi=rjmp.2012.123.135

[16] http://idosi.org/abr/1(1-2)/2.pdf

If we look to the standard errors of these two bacteria and plants, standard error of antibacterial effect of *Chichorium intybus* on *Escherichia coli* is 0.3 and standard error of antibacterial effect of *Urtica dioica* on *Escherichia coli* is 0.4. These standard errors are too small values. This is important because they mean the errors in the experiment. If the errors are too small in an experiment, that means the experiment went consistent and the results are relative. Same thing is valid for standard errors of antibacterial effects of plants on *Staphylococcus aureus.* Standard error of antibacterial effect of *Chichorium intybus* on *Staphylococcus aureus* is 0.3 and standard error of antibacterial effect of *Urtica dioica* on *Staphylococcus aureus* is 0.3.

My hypothesis *Chichorium intybus* and *Urtica dioica* both have significant antibacterial effect on both gram positive and gram negative types of bacteria is supported by results and data analysis of this extended essay experiment.

Another important point is in data analysis; that standard error is small. This shows that, error is minimum. While standard error is increasing, errors in the experiment increase too. It is significant to have lower standard error is important.

During the experiment, there were some unexpected conditions, which could affect the results. Accuracy of measuring apparatus, using a ruler which has more scale degree. Limitations of the experiment method, things which cannot be done in that lab.

Daily and seasonal changes during picking the plants can affect the physiological development of plants. This can be another source of error. This problem can be another essay's subject. Researchers can do the same experiment in different seasons and observe the differences.

If I used Hektoen Enteric Agar instead of Mueller Hinton Agar, I might not reach the same results which I reached by using Mueller Hinton Agar because Hektoen enteric Agar prevents the growth of gram positive bacteria. So that would be a problem for me for measuring the real inhibition zone values while using an Agar which prevents the growth of *Staphylococcus aureus.* Same thing is valid

for using Eosin methylene blue Agar because it prevents the growth of gram negative bacteria. So the same problem is valid for this Agar which prevents the growth of *Escherichia coli.*[17]

[17] http://faculty.mdc.edu/jmorata/TYPES%20OF%20AGAR.pdf

VIII. CONCLUSION

My research question was “How does the antibacterial effect of *Chichorium intybus* and *Urtica dioica* change on different types of bacteria *Staphylococcus aureus* and *Escherichia coli*?” This question is answered by my experiment and its result. Different plants can show different antibacterial effects on different types of bacteria. *Chichorium intybus* gives more antibacterial reaction to gram positive bacteria *Staphylococcus aureus* than *Escherichia coli*. It also gives more antibacterial reaction to gram positive bacteria *Staphylococcus aureus* than *Urtica dioica.* But two types of plant give the equal reaction to the gram negative bacteria *Escherichia coli.*

Different plants give different antibacterial reactions to different bacteria and inside of the plant headline, antibacterial property can differ from part to part. For this extended essay, I could not do all plants and all parts of different plants so subject is narrowed to two plant and two bacteria.

This extended essay can be a source for researchers and new researches that will be done in future about antibacterial effect of different types of plants and different parts of plants. Researchers can use my experiment’s results in their researches for investigating antibacterial effects of different plants on some type of bacteria. They can determine new plants which have antibacterial effect on only gram positive or gram negative bacteria. Researchers can also investigate the gene that is creating these antibacterial properties. In future studies researchers can transfer this gene to other plants and investigate the effect of this gene on other plants' antibacterial properties.

To sum up, these plants whose antibacterial effect is determined can be used as a source of new chemotherapeutic s’ synthesis. I think in future, in the medicine and pharmacy sector, using of these plants in the production of medicines which are less harmful to organism, will be good and these values which found on this experiment can help the choice of plant in the situations which needs antibacterial intervention.

People can battle with bacteria, illnesses which caused by bacteria by the help of natural techniques instead of medicines by these plants which have antibacterial effects.

IX. APPENDICES

A) Appendix I

The information which is given below is about Mueller Hinton Agar which used for a medium for *Staphylococcus aureus* and *Escherichia coli.*

"Intended Use

Mueller Hinton Agar is used in antimicrobial susceptibility testing by the disk diffusion method. This formula conforms to Clinical and Laboratory Standard Institute (CLSI), formerly National Committee for Clinical Laboratory Standards (NCCLS).

Product Summary and Explanation

Mueller Hinton Agar is based on the formula recommended by Mueller and Hinton for the primary isolation of Neisseria species. Mueller and Hinton selected pea meal extract agar as a simple transparent medium containing heat stable ingredients. During their modification, starch replaced the growth-promoting properties of pea extract, acting as a "protective colloid" against toxic substances. Bauer, Kirby, Sherris and Tuck recommended Mueller Hinton Agar for performing antibiotic susceptibility tests using a single disk of high concentration. This unsupplemented medium has been selected by the Clinical and Laboratory Standard Institute (CLSI) for several reasons. This medium is low in sulfonamide, trimethoprim and tetracycline inhibitors, and provides satisfactory growth of most non-fastidious pathogens along with demonstrating batch-to-batch reproducibility. Mueller Hinton Agar is often abbreviated as M-H Agar, and complies with requirements of the World Health Organization. Mueller Hinton Agar is specified in FDA Bacteriological Analytical Manual for food testing, and procedures commonly performed on aerobic and facultatively anaerobic bacteria. A variety of supplements can be added to Mueller Hinton Agar, including 5% defibrinated sheep or horse blood, 1% growth supplement and 2% sodium chloride.

Principles of the Procedure

Beef Extract and Acid Hydrolysate of Casein provide nitrogen, vitamins, carbon, and amino acids in Mueller Hinton Agar. Starch is added to absorb any toxic metabolites produced. Agar is the solidifying agent. A suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and inner zonal growth,

is demonstrated on medium possessing high levels of thymide. The PABA and thymine/thymidine content of Mueller Hinton Agar are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Formula / Liter

Beef Extract ..... 2 g

Acid Hydrolysate of Casein..... 17.5 g

Starch..... 1.5 g

Agar ..... 17 g

Final pH 7.3 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications."[18]

[18] http://www.neogen.com/Acumedia/pdf/ProdInfo/7101_PI.pdf

B) Appendix II

The information which is given below is about Mc Farland Standards which bacteria are set to Mc Farland Standards in the experiment.

"PURPOSE:

McFarland standards provide laboratory guidance for the standardization of numbers of bacteria for susceptibility testing or other procedures requiring a standardization of the inoculum. A 0.5 McFarland standard is comparable to a bacterial suspension of 108 cfu/ml.

PRINCIPLE:

After the discovery of sulfonamides and penicillin, patients were treated empirically and the organisms were mostly susceptible. Emergence of resistance occurred, requiring that organisms be tested in vitro against antimicrobial agents. For many types of susceptibility testing, a standard inoculum of bacteria must be used. McFarland standards were devised to replace the counting of individual cells and are designed to correspond to approximate cell densities as required by the method of antimicrobial testing.

FORMULAS:

(1) 0.5 McFarland Standard: *item no. R6540*

Sulfuric Acid, 1%..... 995.00 ml

Barium Chloride, 1%..... 5.00 ml" [19]

[19] http://www.pmlmicro.com/assets/TDS/500.pdf

C) Appendix III

The information which is given below is about spread technique which used for preparing agar with *Staphylococcus aureus* and *Escherichia coli.*

"The purpose of the spread-plate technique is to grow and isolate colonies of bacteria. A sample of bacteria is transferred to the agar plate, an environment that provides nourishment for the bacteria to grow. The bacteria sample is applied to the agar plate which a special streaking technique that dilutes the amount of bacteria in each section of the agar plate continuously. This is because if you just swabbed the bacteria onto the plate with no special technique the colonies would grow very densely together and be difficult to study. The streaking technique gradually dilutes the amount of bacteria in each 'quadrant' of the plate, so the last quadrant should have small, isolated colonies that can be easily studied.

The spread plate technique is also used for the enumeration of aerobic microorganisms from the given sample. This can be done by serial diluting the samples, placing 0.1ml of the diluted sample in the middle of an agar plate and spreading the sample over the surface with a help of an L-rod. After the incubation rhe colonies can be counted." [20]

[20] http://wiki.answers.com/Q/What_is_the_purpose_of_the_spread-plate_technique

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