INTERNATIONAL BACCALAURETE BIOLOGY EXTENDED ESSAY

Investigating the antibacterial effect of *Camellia sinensis (*Tea/Green Tea) on *Escherichia coli (some types causes skin damage and have similar properties of bacteria casuing acnes)* for the alternate acne medication.

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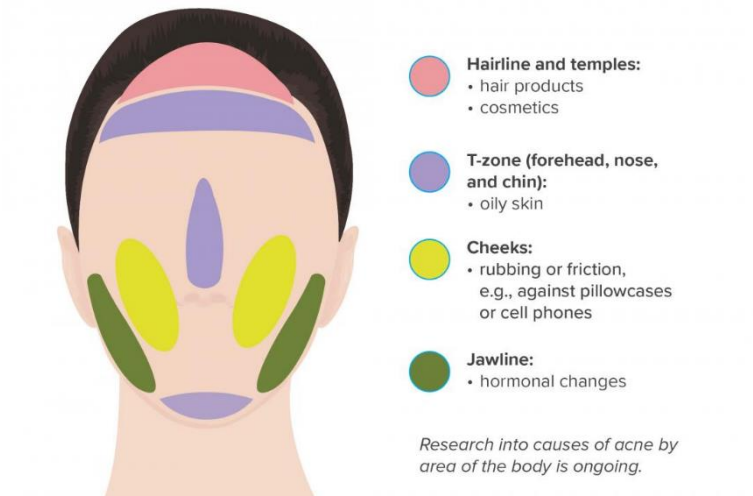
INTRODUCTION

Due to hormonal changes and stress, acnes have started to appear on my face. However, many years before, my skin was balanced and acne or pimple-free, even when my period had started which is usually a big hormonal development and the beginning of imbalance on the bodies for young females.[1] Not only does teenage body start to grow, their skin types become more certain as well as visualize hormonal changes more clearly and actively.

According to my doctor, the main trigger for me was stress and life-style adaptations. Other than high-school has added stress, the isolation due to COVID-19 has weakened my immune system and brought some major changes on my skin. The locations of acnes have also provided additional evidence. For example, if the location is forehead, it is usually caused by sleep deprivation, stress, and liver problems or cosmetics.[2]

Figure 1: Image into causes of acne by are of the body is ongoing.



The treatment process for acne-prone skin includes many medications which I tried some consisting antibiotics prescribed by dermatologists. However, in the long term, using only and so many drying medications did not work as the overlydried areas irritated sebaceous glands to produce more oil, which resulted in constant cycle of acne growth. In addition, antibiotics or

[1] Herndon, Jaime. "No Menstruation (Absent Menstruation)." Healthline, 29 May 2020, www.healthline.com/health/menstruation-absent#take-action.

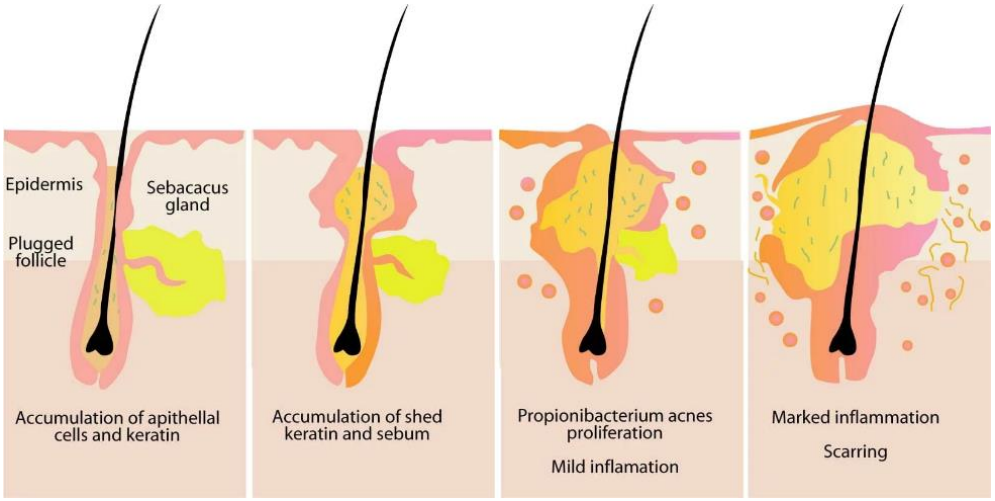
[2] Andriakos , Jacqueline. "Acne Face Mapping Can Reveal the True Cause of Your Breakouts." *Health.com*, Jacqueline Andriakos, 10 Jan. 2017, https://www.health.com/condition/acne/acne-face-mapping.

medication are only able to heal the vulgaris and eliminate the amount but cannot give 100% solution to pimples, especially to the young females and males in active hormone years. It is even known that bacteria develop resistance to these antibiotics by time.[3] For these reasons, dermatologists' advice skincare routines to keep the oil production under control. Due to these, I have started my research about how I can decrease chemical use and focus on more natural products. By this, I have gathered information about what causes acne, what is acquired by the skin for prevention and which natural plants might help.[4]

Acne

Acne is a skin condition when the hair follicles are clogged up with dead skin and oil that helps bacterial growth.[5] Generally, the types differ from skin types, and what has grown *(ex: blackheads, pimple, etc.).* Acne symptoms can also vary depending on its type and activeness. It is most common in children during puberty, including 80% of people between the ages of 11 and 30 have had at least one acne problem.[6]

Figure 2: Image about active acne vulgaris production process under-skin.



[3] Team, Children's Health. "Antibiotics for Acne: How Much Is Too Much?" *Cleveland Clinic*, Cleveland Clinic, 29 Oct. 2021, https://health.clevelandclinic.org/antibiotics-for-acne-how-much-is-too-much/. , Dao, Minh, et al. "Potential Harms of Long-Term Acne Treatment with Oral Antibiotics." *Canadian Family Physician Medecin De Famille Canadien*, College of Family Physicians of Canada, Sept. 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7491659/.
[4] Team, Children's Health. "Antibiotics for Acne: How Much Is Too Much?" *Cleveland Clinic*, Cleveland Clinic, 29 Oct. 2021, https://health.clevelandclinic.org/antibiotics-for-acne-how-much-is-too-much/.
[5] Staff, Mayo Clinic. "Acne." *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 12 Sept. 2020, https://www.mayoclinic.org/diseases-conditions/acne/symptoms-causes/syc-20368047.
[6] Clinic, Claveland. "Acne: Treatment, Types, Causes & Prevention." *Cleveland Clinic*, Claveland Clinic, 9 Jan. 2021, www.my.clevelandclinic.org/health/diseases/12233-acne.

The treatments vary, for natural products. However, in recent years, most used natural substances are tea tree oils. This oil is found as anti-bacterial similarly aloe vera, jojoba oil, green tea…[7] Yet in recent products, the percentage of extraction of green tea has been the most preferred for skincare and medications. It is because tea, green tea in specific, consist of different types of "polyphenols," which are antioxidant compounds with antimicrobial, anti-inflammatory, and antineoplastic properties.

This research has made me question whether this everyday consumed substance: green tea really helps reducing the activation of bacteria for sebum production. If so, does the mass of green tea really affect the result?

At the same time, due to working with bacteria is easy as they reproduce very quickly, I chose *Escherichia coli*, a commonly found type in animal bodies and have similar properties as some skin inflammatory bacteria. In fact, some people have shown to grow skin infections due to *E.coli*.[8] Due to safety restrictions, main bacterium types that cause inflammatory effects on skin such as *Propionibacterium* which is a gram-positive bacterium with thicker cell walls compared to gram-negatives and slow reproducing type cannot be chosen for my experiment. Form many studies, gram-positive bacteria are considered to be in a high-risk group of pathogenic microorganisms.[9] In comparison, *E.coli* is a gram-negative bacteria which has similar properties as bacteria causing gram-negative folliculitis that are pustular rashes by acne treatment processes. Also, its pathogenicity is not as high and easier to observe in laboratories, making it safer to use.

[7] Huizen, Jennifer. "Top 15 Home Remedies for Acne." *Medical News Today*, MediLexicon International, 13 July 2018, www.medicalnewstoday.com/articles/322455#home-remedies.
[8] Sunder, S et al. "Life-threatening Escherichia coli cellulitis in patients with haematological malignancies." *Journal of medical microbiology* vol. 61,Pt 9 (2012): 1324-1327. doi:10.1099/jmm.0.042366-0
[9] Canada, Public Health Agency of. "Government of Canada." Canada.ca, / Gouvernement Du Canada, 30 Apr. 2012, https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/escherichia-coli-enteropathogenic.html.

To have the most effective results, my research question is *"How do different masses of (0.5 g/ 2.0 g/ 4.0 g/ 6.0 g/ 8.0 g) of extract of green tea leaves from Camellia sinensis affect the colony number of Escherichia coli planted in petri dishes for 24 hours & counted as viable cells?"*

HYPOTHESIS

To recent days there has been more than 8,000 polyphenol types discovered naturally occuring in plants.[10] According to recent research, polyphenols are found to be possibly beneficial in alternative treatments against sebum-oily substances produced by sebaceous glands- and acne vulgar treatments.[11] Since polyphenols are beneficial in vulgar treatments as well as in skincare; green tea extracts which are rich in a variety of ploy phenol types, will be beneficial. As the mass of *Camellia sinensis* increases colony number of *E.coli* decreases by the help of home-produced acne treatment.

VARIABLES

I. Independent Variables

In the investigation, concentration of green tea extract is the independent variable. The mass of green tea used with ethanol (%25) and distilled water (%75) solution (each to prepare the extracts) are 0.5 g, 2 g, 4 g, 6 gr, 8 g (± 0.01 gr). For each investigation 20 drops (0.5 ml for each drop) (±0.006 ml) of extract is used.

[10] Pandey, Kanti Bhooshan, and Syed Ibrahim Rizvi. "Plant polyphenols as dietary antioxidants in human health and disease." Oxidative medicine and cellular longevity vol. 2,5 (2009): 270-8. doi:10.4161/oxim.2.5.9498

[11] Saric, Suzana, et al. "Green Tea and Other Tea Polyphenols: Effects on Sebum Production and Acne Vulgaris." Antioxidants (Basel, Switzerland), MDPI, 29 Dec. 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC5384166/

II. Dependent Variables

The dependent variable for the experiment is colony number of *E.coli* at the end of 24 hours of reproduction time.

III. Controlled Variables

Table 1: Controlled variables, their effects on the investigation and methods of control

Controlled Variables	**Effect on the Experiment**	**Methods for Control**
Incubation time	*E.coli* can multiply each 20 minutes under lab conditions when the environment provides enough source.[12] For this reason, when they are left in longer time periods, effects can be determined more clearly.	Petri dishes with blood agar are left 24 hours in laboratory incubator mechanism, under sterilized and isolated conditions.
E. coli strain	Different *E.coli* strains may result variously for each investigation.	*E.coli K-12* is used as the strain for every petri dish which is non-pathogenic. The colonies are taken from the initial laboratory sample and divided for each trial.
Incubation temperature	Temperatures below the optimum (37 °C) degree for *E.coli* bacteria may slower the growth as well as the degrees increase.[13] In addition, different temperatures for each investigation will result in false notion.	As the optimum temperature is 37 °C for *E.coli*, it can grow between 30 °C and 42 °C with different rates[14]. For the experiment, all petri dishes were kept under 37°C environment in incubators.

(cont.)

[12] Gibson, Beth et al. "The distribution of bacterial doubling times in the wild." Proceedings. Biological sciences vol. 285,1880 (2018): 20180789. doi:10.1098/rspb.2018.0789

[13] Tuttle, Amie R et al. "Growth and Maintenance of Escherichia coli Laboratory Strains." Current protocols vol. 1,1 (2021): e20. doi:10.1002/cpz1.20

[14] Doyle, M P, and J L Schoeni. "Survival and growth characteristics of Escherichia coli associated with hemorrhagic colitis." Applied and environmental microbiology vol. 48,4 (1984): 855-6. doi:10.1128/aem.48.4.855-856.1984

Technical equipments	The apparatus allows bacteria to be planted on petri dishes, solutions prepared, and bacteria being put in the testing tubes all equally for more reliable results.	The testing tubes, the 3 ml pipettes (1 ml drop), disposable inoculation loops (used once and own individual for every solution mixed with bacteria), petri dishes with blood agar (better nutrients for *E.coli*) are all identically used in trials.
Sterilization and Lab conditions	The bacteria used can contaminate and lab is used for all microbiology included purposes which non-sterilized apparatus will/may change the results and reliability.	Every equipment and technical apparatus used has been opened from their packages *(bought in sterilized form)* for the first time and never been used later *(medical waste)*. The laboratory environment was arranged according to "National Microbiology Laboratory Standards Safety Guidelines". [15]

IV. Materials

Table 2: The quantities and sizes of general equipment and chemical substances needed.

Materials (General & Chemical)	Unit & Size	Quantity
Electronic weighing scale	(±0.01g)	1
Beaker	1000 mL (±5 mL)	1
Jars with caps	150 mL (±1 mL)	5
Beaker	100 mL (±1 mL)	1
Transfer graduated pipettes	0.5 mL drop by (±0.006 mL)	8
Sterilized polystyrene Petri dishes *(with EMB agar)*	17 mm x 80 mm	6

(cont.)

[15] General Directorate of Public Health. "Laboratuvar Güvenliği Rehberi: Laboratory Safety Guide." *https://hsgm.saglik.gov.tr/depo/kurumsal/yayinlarimiz/rehberler/UMS-Laboratuvar_GAveliAi_Rehberi-2021_2_versiyon.pdf*, Ministry of Health of the Republic of Turkey, 2021, hsgm.saglik.gov.tr/depo/kurumsal/yayinlarimiz/rehberler/UMS-Laboratuvar_GAveliAi_Rehberi-2021_2_versiyon.pdf

Labaratory test tubes	-	26
Labaratory water bath	60 ℃ (±0.5℃)	1
Sterilized disposable inoculation loops	-	25
Incubator	37 ℃ (±0.5℃)	1
Plastic testing tube stand	-	1
Plastic gloves	-	4
Stock colony of *E.coli*	200 colony (cfu)	-
Distilled water	235 mL (±1 mL)	-
Dried green tea leaves from *Camellia sinensis*	20.5 g (±0.01g)	-
Ethyl alcohol (%95 ethanol)	75 mL (±1 mL)	-
Filter paper	-	5

V. Sterilization

During these research *National Microbiology Laboratory Standards Safety Guidelines* [16] has to be considered as the most important rule book which for every lab an obligation.

METHOD DEVELOPMENT

Extraction

To work on *E.coli* and its reaction towards medication applied, there is need for a scientific microbiology lab. I have visited the microbiologist Ayşegül Akman in Lokman Hekim Hospital. By her approval, I was able to conduct my experiment in March & April 2022 in her labaratory. *(Appendix I)*

[16] "General Directorate of Public Health. "Laboratuvar Güvenliği Rehberi: Laboratory Safety Guide." *https://hsgm.saglik.gov.tr/depo/kurumsal/yayinlarimiz/rehberler/UMS-Laboratuvar_GAveliAi_Rehberi-2021_2_versiyon.pdf*, Ministry of Health of the Republic of Turkey, 2021, hsgm.saglik.gov.tr/depo/kurumsal/yayinlarimiz/rehberler/UMS-Laboratuvar_GAveliAi_Rehberi-2021_2_versiyon.pdf.

The *C.sinensis(green tea)* were purchased from herbalist in dried fresh leaf form. It was intended that, the fresh leaves without any chemical processes, the extraction was more reliable rather than supermarket teas. The leaves were separated according to their masses via scale and left in ethyl alcohol-distilled water solutions for 3,5 hours. *(explained in method)* The sample concentrations for extractions were chosen to determine the minor differences between control data and 0.5 gram green tea leaf extracted specifically.

In continuation, the mass of leaves increase in balance (2-4-6-8) in order to determine a significant relation between antioxidants extracted and bacterial colony number. During the extraction process, because hot water extraction is chosen for faster progress and tea solutions are left in 60℃ water baths for 3,5 hours till the colour change of tea is darker than normal. This indicates the chemicals are released in alcohol.

The ethyl alcohol and distilled water were purchased from stores with laboratory equipment. And water baths are used in school laboratory after the confirmation of laboratory assistant teacher.

Bacteria Growing on Petri Dish

Everyday microbiological experiment technique is determined to be easier to examine the growth of bacteria in a short amount of time. The agar jelly (blood agar/ tryptic soy agar/ EMB agar) on the petri dishes is rich-substrate with easier allowance to bacteria growth.[17] As it is a solid substance, bacteria remain on top with high range of visibility for better results. In laboratory systems, incubators let the agar with bacteria stay in an isolated and sterilized

[17] Lal, Archana, and Naowarat Cheeptham. "Eosin-Methylene Blue Agar Plates Protocol." American Society for Microbiology, 29 Sept. 2007, https://asm.org/ASM/media/Protocol-Images/Eosin-Methylene-Blue-Agar-Plates-Protocol.pdf?ext=.pdf. Accessed 2022.

environment (arranged according to the aimed bacteria for its best conditions) for the needed time.[18]

The transfer of bacteria is done with inoculation loops, rather than pour plate technique. While *E.coli* increases its numbers fast, the pouring technique generally results with greater bacteria colony numbers, hardening the counting process. Inoculation loops do control the number of bacteria transferred and the area it can pour and grow into, without releasing the complete bacteria solution on petri dishes.

E.coli is a fast growing bacteria type, so it is easier to work on with. Which is why, stocked 200 cfu *E.coli* colony was used after diluted by factor 10 and mixed with extraction samples via droppers.

Colony Calculation

Figure 3: Picture of the colour and shape of E.coli bacteria after incubation. (self-taken)

I chose CFU/ ml formula for the calculation of colony number. Cfu (colony forming unit) is a measuring system that is used only for the viable bacteria cells. For the calculations of liquid samples of bacteria, formula *cfu/ ml (colony forming unit per ml)* is preferred.[19] Before my experiment, the bacteria colonies are taken from a petri dish to testing tube, where it is distilled with 10 mL water. For calculations, the distillation ratio is also considered.

[18] Tarun Madappa, MD. "Escherichia Coli (E Coli) Infections Workup." Laboratory Studies, Imaging Studies, Other Tests, Medscape, 17 Oct. 2021, https://emedicine.medscape.com/article/217485-workup#:~:text=E%20coli%20is%20a%20gram,E%20coli%20strains%20are%20nonpigmented.

[19] "CFU: Colony Forming & Calculation." 21 Nov. 2011, http://technologyinscience.blogspot.com/2011/11/cfu-colony-forming-unit-calculation.html. Accessed 2 Dec. 2022.

Because the technical machine was not available in the labaratory, the green-coloured colonies formed on agar are counted according to their labeled areas by hand from the back of the dishes. A formula is used to calculate cfu/ml.

Agar Preparation

Since *Methylene Blue Agar (EMB)* is already prepared within petri dishes, there was no preparation for petri dishes separately.

During experiment laboratory equipments such as petri dishes, loops, testing tubes and etc. were purchased by the laboratory, which was allowed for me to utilize.

For more reliable research, 5 trials were strived for each extraction sample including the control samples, which sum up 30 trials were performed as a gross total of experiment.

Water Baths

The optimum conditions for extraction method is 80℃ for 30 minutes for 10g green tea leaves. Fort he experiment, the mass of green tea leaves being extracted are smaller, which is why the temperature arranged is 60℃ in lab water baths while the time is chosen according to it as 3,5. In experimenting, the temperature can be arranged according to water bath conditions, meanwhile the time must be calculated inversely to the celcius degree.[20]

Extraction Method

1. Mix the distilled water and ethyl alcohol *(contains approximately %95 ethanol)* according to 15 mL ethanol and 45 mL distilled water (± 0.01 ml) ratio for 5 jars (60 mL for each) with a closed cap to prevent ethanol evaporation.

[20] Vuong, Quan & Stathopoulos, Costas & Golding, John & Nguyen, Minh & Roach, Paul. (2011). Optimum conditions for the water extraction of L-theanine from green tea. Journal of separation science. 34. 2468-74. 10.1002/jssc.201100401.

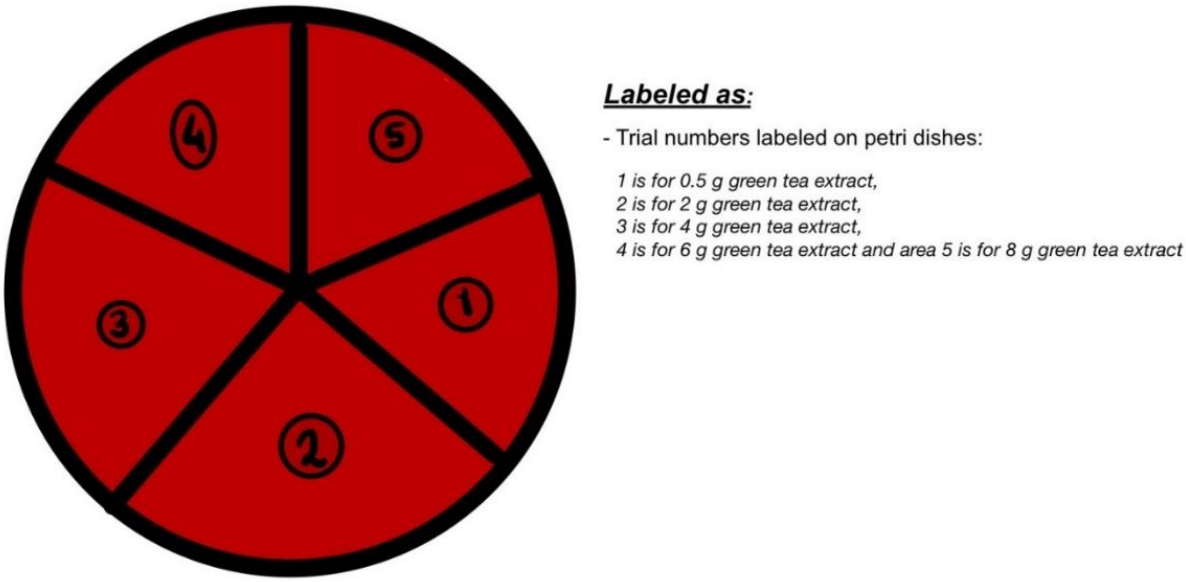
2. Arrange the temperature of the laboratory water bath to 60℃, weigh tea leaves according to 0.5 g/ 2 g/ 4 g/ 6 g/ 8 g ± 0.01 g mass ratios and put them into the jars one by one. Stir the leaves with ethyl alcohol and water solution prepared in step 1 until all the leaves are wet.
3. Close the caps to prevent the ethanol evaporation. Put the jars into water-baths for 3,5 hours.
4. After 3,5 hours take the jars away from the water baths and after visually determining the colour change in ethyl alcohol solutions, let the jars cool to minimize the amount of water vapor and alcohol before opening the cap. When the temperature of the jars are equal with the room temperature (20℃) open the caps and filter the leaves of green tea from the extractions with the help of a filter paper and put the extraction into the jars again with closing the cap.
5. Keep the extractions in room conditions without opening the jars until experiment time.

Sample Preparation

6. Add 10 mL of distilled water with transfer graduated pipette *(Appendix II-Figure-2)* to one of the testing tubes. Get 200 cfu (colony) *E.coli* from a stocked sample in a petri dish with inoculation loop and stir with the distilled water solution in the testing tube. After mixing, put the tube on to the tube stand *(Appendix II-Figures-1,3)*.
7. Take one testing tube and label it as 0.5g. Add 10 mL extract of 0.5 g green tea leaves.
8. With another transfer pipette, add 3 drops (≈ 1.5 ml) of *E. coli* + distilled water mixture from the tube prepared.
9. Repeat steps 8-9 for 2/4/6/8 g green tea leaves extracted.

Petri dishes with same mass of EMB agar as shown in figure 4.

Figure 4: Self made model of distribution of petri dish and labeling.



Because the laboratory was not able to provide plastic separator pieces for petri dishes due to technical problems, we were left decided on labeling with the help of the microbiologist.

10. Once all the petri dishes are done with labeling, inoculate each with mixture of E. coli extraction solution, by using the loops *(Appendix II-Figure-6)*. First from the middle of the petri plate (bottom end of the triangle areas) draw a straight line to the top and then move with zigzag shape from one side to the other (without touching the division lines) to the bottom of the shape to evenly distribute.
11. Repeat steps 8-11 for 4 more times to have 5 trials for all mas ratio.
12. In one of the petri dishes inocluate *E.coli* and distilled water solution for the control data.
13. Put all samples' covers and put them inside the laboratory incubators at 37°C. *(Appendix II-Figure-5)*

Figure 5: Self-taken picture of testing tubes on tube stand after addition of bacteria and extracts.

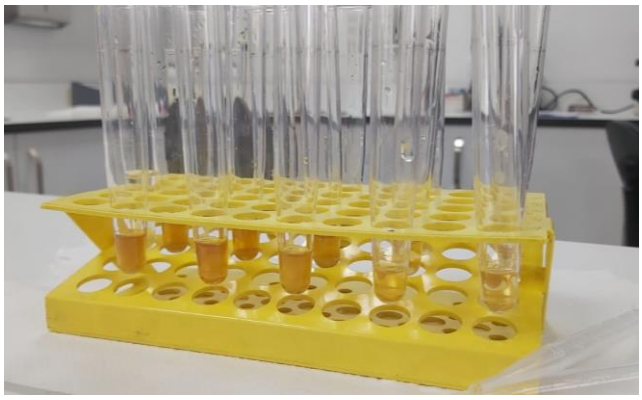
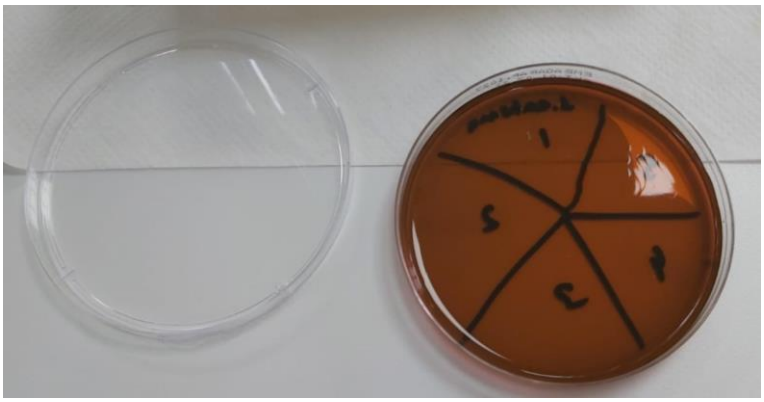


Figure 6: Self-taken picture of first labeled trial's inoculated petri dish before putting into incubation.



14. After 24 hours of incubation at 37℃, the petri dishes are taken out and count the colonies which are generally stay in visible dot shapes with green metallic colour covering their surroundings.
15. Count the colonies by marking from the back of petri with a pen, according to labeled areas.
16. Use formula to reach the cfu/ml calculated number of viable bacteria, do the statistical analysis to find the relation.

DATA COLLECTION

I. Qualitative

The shapes and visible colonies are considered as the qualitative/ initial data gathered before quantitative is acquired. The colony number are more visible and crowded in the area labeled as “1” according to the trials. However because the divisions are put as labels on the petri dishes, for some trials *E. coli* colonies have reproduced towards and even some directly into the othered labeled parts which caused false and unexpected results.

According to this figure, number one where only 0.5 g of green tea leaves were used and extract is expected to be with the highest number of bacterial colony. Results have reached the expectation while clearly most crowded is the "1" labeled area. The results have also reached the expectations in area number 2 while the number of bacteria are seemed to be second highest in here. The fifth area's expectation was it having similar or less colonies than area 3. In control plate however, the colony number is higher at every area, while the extractions were not inoculated on.

Figure 7: Image of the 1st trial which had resulted in mistaken results because of the movement of E. Coli bacteria.

II. Quantitative

Table 3: The number of viable E.coli colony numbers according to the gram use of Camellia sinensis for extraction after 24 hours at 37℃ incubation.

	Number of E. coli colonies						Controlled Variables	
Trials	0-gram tea extract (±0.01g)	0,5-gram tea extract (±0.01g)	2-gram tea extract (±0.01g)	4-gram tea extract (±0.01g)	6-gram tea extract (±0.01g)	8-gram tea extract (±0.01g)	Incubation Time *Hours (±0.2)*	Incubation Temperature *(℃) (±0.5)*
1	193.00	142.00	65.00	41.00	27.00	17.00	24.0	37.0
2	201.00	129.00	71.00	33.00	31.00	22.00		
3	208.00	147.00	64.00	43.00	30.00	21.00		
4	197.00	131.00	62.00	44.00	25.00	19.00		
5	190.00	126.00	69.00	38.00	28.00	18.00		

This calculation of colonies are done with counting the visible green small groups which are the colonies that are active for each. Because there was no bacteria counting machine and that they count non-living bacteria as well, I have chosen to use a formula and calculation to measure the approximate number of bacteria cells although each colony may differ by cell number.

Table 4: The graph of calculated E.coli viable cells according to the formula given.

	Colony Forming units – CFU/mL *E. coli*						Controlled Variables	
Trials	0-gram tea extract	0,5-gram tea extract	2-gram tea extract	4-gram tea extract	6-gram tea extract	8-gram tea extract	Incubation Time (hours) *(±0.2)*	Incubation Temperature (°C) *(±0.5)*
1	1286	946	433	273	180	113	24.0	37.0
2	1340	860	473	220	207	146		
3	1353	980	427	286	200	140		
4	1313	873	413	293	167	126		
5	1266	840	480	253	186	120		

The formula:

$$Number\ of\ Viable\ Cells\ per\ mL = \frac{Number\ of\ colonies\ (CFU) \times Dillution\ Factor}{Volume\ of\ culture\ plate\ (mL)}$$

Ex: (First trial of 0.5 g green tea extraction)

Number of colonies: 142 cfu

Dilution factor: 10^1

Volume of Culture plate: 1.5 mL *(3 drops equals to 1.5 mL of 10 mL distiled water + E. Coli is added to each testing tubes which results in 1.5 mL volume of incubation)*

$$Number\ of\ viable\ cells = \frac{142\ cfu\ \times 10^1}{1{,}5\ mL} = 946{,}6666 \ldots \approx 946{,}6$$

III. Statistical Calculations

Mean

$$\overline{\boldsymbol{x}} = \frac{\sum_{i=1}^{n} x_i}{\boldsymbol{n}} = \frac{\boldsymbol{Sum\ of\ all\ data\ points}}{\boldsymbol{number\ of\ data\ points}}$$ where;

n is the number of trials in the groups

x is the dissolved oxygen level of *Elodea canadensis*

Example: $\frac{(1286{,}66)+(1340)+(1353{,}33)+(1313{,}33)+(1266{,}66)}{5} = 1312{,}00$

Standard Deviasion

$$\boldsymbol{\sigma} = \sqrt{\frac{\sum_{i=1}^{n}(x_i-\overline{X})^2}{\boldsymbol{n}}}$$ where**,**

n is the number of trials in the groups

x is the dissolved oxygen level of *Elodea canadensis*

$\boldsymbol{x}^{-}$ is the mean value of the trial group

Example:

$$\sqrt{\frac{(1286{,}66-1312{,}00)^2+(1340-1312{,}00)^2+(1353{,}33-1312{,}00)^2+(1313{,}33-1312{,}00)^2+(1266{,}66-1312{,}00)^2}{5}}$$

$$\cong 36{,}02697$$

Standard Error:

$$\boldsymbol{SE} = \frac{\boldsymbol{\sigma}}{\sqrt{\boldsymbol{n}}}$$ where,

σ is the standard deviation

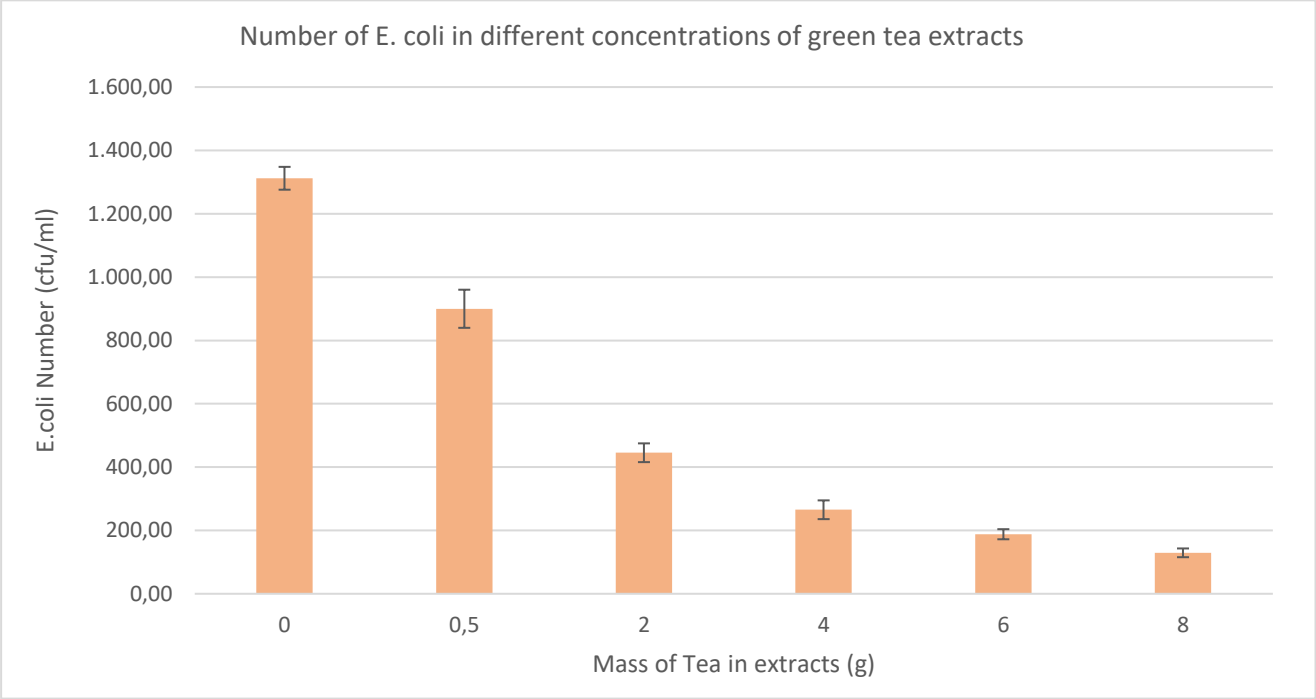
n is the number of samples

Example: $\frac{36,02696532}{\sqrt{5}} = 16.1117487$

Table 5: The graph of calculated mean, standard deviations and standard errors of different conculison levels.

	0-gram tea extract	0,5-gram tea extract	2-gram tea extract	4-gram tea extract	6-gram tea extract	8-gram tea extract
Mean	1312,00	899,99	445,33	265,33	187,99	129,33
St. D	36,02	60,18	29,59	29,58	15,91	13,82
St. Error	16,11	26,91	13,23	13,23	7,11	6,18

Graph 1: Mean of the number of E. coli bacteria in different concentrations of green tea extracts in cfu/ml with error bars according to standard deviation calculations.



IV. ANOVA Tests

ANOVA, analysis of variance, is a method dividing observed data into different groups of two or more in order to determine whether there are significant differences/relation between the groups. The ANOVA type used in this analysis is the “Single Factor Test” which determines

the differences between calculated raw data values of three or more diverse groups.[21] To have done the test for my observations, the p-value has to be statistically significant which $p < 0.05$ so that my hypothesis can be accepted and null hypothesis can be rejected.

H_0: There is no statistical difference between the mass of green tea leaves and number of *E.coli* colony reproduced.

H_1: There is statistical difference between the mass of green tea used in extract and number of *E.coli* colony reproduced.

Table 6: ANOVA-Single Factor Test for Raw Data Table by Excel.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	5	6559,98	1311,996	1297,942
Column 2	5	4499,99	899,998	3622,111
Column 3	5	2226,65	445,33	875,6445
Column 4	5	1326,65	265,33	875,4445
Column 5	5	939,98	187,996	253,3467
Column 6	5	646,65	129,33	191,0889

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5512695	5	1102539	929,6834	1,21E-26	2,620654
Within Groups	28462,31	24	1185,93			
Total	5541158	29				

[21] "One-Way ANOVA." *One-Way ANOVA - An Introduction to When You Should Run This Test and the Test Hypothesis | Laerd Statistics*, https://statistics.laerd.com/statistical-guides/one-way-anova-statistical-guide.php.

By the ANOVA test results, the P-value of the samples, P-values at 5% significance level, are determined very small ($1{,}21.10^{-26}$) Therefore, we reject the null hypothesis which meanst there is significant difference between mass of green tea and number of *E.coli* colonies.

The given ANOVA test doesn't identify the specific relations and differences between raw data trial values. Which is why, I used Post Hoc Tukey's test to analyze the relation among values of groups. Because Excel doesn't have the feature, I used a website: https://astatsa.com/OneWay_Anova_with_TukeyHSD/

V. Tukey HSD results

Data entered according to group A is values of "0-gram tea extract", B is "0,5-gram tea extract", C is "2-gram tea extract", D is "4-gram tea extract", E is "6-gram tea extract" and F is "8-gram tea extract".

Table 7: Post-Hoc Tukey's Analysis

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
A vs B	26.7517	0.0010053	** p<0.01
A vs C	56.2739	0.0010053	** p<0.01
A vs D	67.9616	0.0010053	** p<0.01
A vs E	72.9830	0.0010053	** p<0.01
A vs F	76.7923	0.0010053	** p<0.01

(cont.)

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
B vs C	29.5223	0.0010053	** p<0.01
B vs D	41.2100	0.0010053	** p<0.01
B vs E	46.2314	0.0010053	** p<0.01
B vs F	50.0406	0.0010053	** p<0.01
C vs D	11.6877	0.0010053	** p<0.01
C vs E	16.7091	0.0010053	** p<0.01
C vs F	20.5184	0.0010053	** p<0.01
D vs E	5.0214	0.0179222	* p<0.05
D vs F	8.8307	0.0010053	** p<0.01
E vs F	3.8093	0.1136642	insignificant

For all group comparisons which are indicated before the graph, the *p-values* refer to a figure higher than the critical level of 1% indicating the significancy. The only insignificant difference determined is between E and F (6-gram green tea & 8-gram green tea).

ANALYSIS

As I intended to discover the impact of polyphenols on bacteria cells, I have used different concentrations of green tea extracts. When data is analyzed, viable bacteria colonies have shown drastic falls as the polyphenol numbers increased. Each extraction sample contained 3 drops of dilluted *E.coli* solution. After 24-hours, each sample visualized reduction in number of bacterial colonies as expected in the hypothesis. However when the mean CFU/mL calculations in Table 5 are compared, the rate of change slowed down after 2 gram of tea leaves contained extraction, In addition, with only 0.5 g-difference, the means have dropped from 1312.00 to 899.00 CFU/mL. However between 4-gram and 6-gram leaves samples, the values have dropped from 265.33 to 187.99 CFU/mL.

In general, the steepest reduction rate is between the data of 0,5-gram and 2-gram leave samples. Although the gram difference between 0.5 g and 2 g leaf containing samples is not wide, difference in bacterial colony numbers is the second highest. This analysis can interpret that, the polyphenols in green tea can reduce the number of bacteria until the maximum capacity of the extraction solutions are reached.

For post-hoc Tukey HSD results, the p-values between each group have proven the outcome and hypothesis, except for the relationship between groups E and F, which are identified as 6-gram and 8-gram extracts. The distinction between these two groups is, after a specific mass number of tea leaves there is no difference in the impact and chemical release of polyphenols in the 60 mL ethanol-water solution. This level can also be defined as the capacity of solution, which has resulted in modest fluctuations in the bacteria colony number between trials.

The standard deviations of the data through all the investigations diverse between *E.coli* colonies obtained after 24 hours. When comparing statistic variables according to their standard

deviations, smaller the values get, the closer the results are to the mean values indicating more precise and accurate results. When the values increase, and the ratio between mean and standard deviation also rises, which the results may spread out. Such as; 29.59129 is the calculated standard deviation for the mean 445.33 cfu/mL from 2-gram sample, which the ratio is near 6,64%. The results overlap with the hypothesis 1, as the polyphenols mostly in green tea do slow down the reproduction of bacteria.

Graph-1 also displays the distinction between mean numbers and standard deviations as error bars. The reduction level in the first three extraction concentrations are visibly high, but after 2-gram tea extractions, the decrease level drops and the differences between bacteria colonies, indicating that after a certain concentration, the antimicrobial affect on *E.coli* bacteria is not as efficient as small numbers of tea leaves.

As error bars, every result can be identified as accurate, while the biggest fluctuations can be seen between 0.5-gram and 4-gram green tea leaves extractions, where the mean change is more visible.

At the same time, the null hypothesis about no difference between the increasing green tea mass and change in colony number of *E.coli* is rejected and hypothesis 1 is accepted. While the p-value is determined less than critical number of 0.05. Hence, the Tukey analysis showed the significant differences among the means of groups, except the last two observations.

EVALUATION

Strengths	**Reason**
Trials and Materials	Having total of 5 trials for each set of green tea extract including the control variables for growing bacteria in EMB agar, reasons for precise results.

(cont.)

Statistics and Analysis	ANOVA and post hoc Tukey's tests are precise and accurate.
Laboratory	The microbiology laboratory is located in a known hospital, and equipment used are obtained from the resources of laboratory.
Viable Cells	Instead of using laboratory technical device, all colonies are counted by hand for 5 times, resulting in accurate results while all coincident and viable colonies were included.

The experiment in general can be considered as successful, and reliable according to the techniques chosen, still there are some flaws which can be considered to improved.

Weaknesses	**Reason**	**Suggestions**
Colony Counting	*E.coli* colonies are counted by hand, simple errors can occur unintentionally.	*E.coli* colonies can be counted under a microscope.
Methodology	Petri dishes were not divided by plastic materials resulting in errors by bacterial movement. At the same time, dishes were kept face down while bacteria have mixed with other extracts although added with inoculating loops.	Each sample group can be done to the same petri dish with divided labeled areas, or plastic pieces for division can be obtained.

(cont.)

Inoculating Loop Technique	The applying process of inoculation loop has its technique, but simple errors due to hand application about how strong or how precise in shape the loop is used each time can occur.	Inoculation can be performed by pouring all the solution on the petri dishes after arranging the volume without physical interference.
Bacteria Number Predictions	Colony counting was done with predicting the cell number in each colony as same, without considering smaller or greater colonies.	More accurate results can be obtained when counting is done under microscope.

CONCLUSION

In conclusion, the experiment and investigation have given an acceptance to the research question: *How do different masses (0.5 gr/ 2 gr/ 4 gr/ 6 gr/ 8 gr) (± 0.1 gr) of extract of green tea leaves from Camellia sinensis affect the number of Escherichia coli by caunting bacteria by planting them in petri dishes for 24 hours?*

As expected, green tea can be accepted as effective natural medication against acne or bacteria although it would not completely eliminate the bacterial growth like medications. The anti-microbial and anti-inflammatory properties of its polyphenols support my hypothesis.

Green tea extract can be a home-made acne or vulgar drying treatment for an alternative to antibacterial treatments. It may not be as effective, but in the long term, it could result better

while bacteria have the ability to adapt and gain resistance against antibiotics which most medications consist.

However, ethyl alcohol is required for the medically or scientifically necessary extraction, which after some use can cause mainly skin allergy and blindness when consumed from mouth as well as headaches, nausea and vomiting and lung or respiratory system problems after steady inhaling.[22] Regardless, use of extractions may be the reason of overdryness, itching, redness, peeling and cracking after repeated exposure. Herein, advices from professionals should be taken, according to the skin types. The reduction of drying effect can be achieved by leaving green tea in hot or boiling water for longer periods without using chemical solvents, or by using it zonary to the inflamed area without spreading.

I can refer to a new research opinion where the extracts can be prepared without ethyl alcohol, by only with hot water. Likewise, research question can be revised as: "How do different masses (0.5 gr/ 2 gr/ 4 gr/ 6 gr/ 8 gr) (± 0.1 gr) of extract of green tea leaves extracted by water from *Camellia sinensis* without any alcohol, effect the number of *Escherichia Coli* in CFU/ml after 24 hours?". This can be a safer home-produced medication for the balance of skin in long-term.

[22] "Ethanol (Ethyl Alcohol)." DCCEEW, https://www.dcceew.gov.au/environment/protection/npi/substances/fact-sheets/ethanol-ethyl-alcohol#:~:text=Ethanol%20is%20harmful%20by%20ingestion,tract%20(nose%20and%20throat).

REFERENCES:

1. Herndon, Jaime. “No Menstruation (Absent Menstruation).” Healthline, 29 May 2020, www.healthline.com/health/menstruation-absent#take-action.

2. Andriakos , Jacqueline. “Acne Face Mapping Can Reveal the True Cause of Your Breakouts.” *Health.com*, Jacqueline Andriakos, 10 Jan. 2017, https://www.health.com/condition/acne/acne-face-mapping.

3. Treatment with Oral Antibiotics.” Canadian Family Physician Medecin De Famille Canadien, College of Family Physicians of Canada, Sept. 2020, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7491659/.

4. Team, Children's Health. “Antibiotics for Acne: How Much Is Too Much?” *Cleveland Clinic*, Cleveland Clinic, 29 Oct. 2021, https://health.clevelandclinic.org/antibiotics-for-acne-how-much-is-too-much/. , Dao, Minh, et al. “Potential Harms of Long-Term Acne

5. Staff, Mayo Clinic. “Acne.” *Mayo Clinic*, Mayo Foundation for Medical Education and Research, 12 Sept. 2020, https://www.mayoclinic.org/diseases-conditions/acne/symptoms-causes/syc-20368047.

6. Clinic, Claveland. “Acne: Treatment, Types, Causes & Prevention.” *Cleveland Clinic*, Claveland Clinic, 9 Jan. 2021, www.my.clevelandclinic.org/health/diseases/12233-acne.

7. Huizen, Jennifer. “Top 15 Home Remedies for Acne.” *Medical News Today*, MediLexicon International, 13 July 2018, www.medicalnewstoday.com/articles/322455#home-remedies.

8. Sunder, S et al. “Life-threatening Escherichia coli cellulitis in patients with haematological malignancies.” *Journal of medical microbiology* vol. 61,Pt 9 (2012): 1324-1327. doi:10.1099/jmm.0.042366-0

9. Canada, Public Health Agency of. “Government of Canada.” Canada.ca, / Gouvernement Du Canada, 30 Apr. 2012, https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/escherichia-coli-enteropathogenic.html.

10. Pandey, Kanti Bhooshan, and Syed Ibrahim Rizvi. “Plant polyphenols as dietary antioxidants in human health and disease.” Oxidative medicine and cellular longevity vol. 2,5 (2009): 270-8. doi:10.4161/oxim.2.5.9498

11. Saric, Suzana, et al. “Green Tea and Other Tea Polyphenols: Effects on Sebum Production and Acne Vulgaris.” Antioxidants (Basel, Switzerland), MDPI, 29 Dec. 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC5384166/

12. Gibson, Beth et al. “The distribution of bacterial doubling times in the wild.” Proceedings. Biological sciences vol. 285,1880 (2018): 20180789. doi:10.1098/rspb.2018.0789

13. Tuttle, Amie R et al. “Growth and Maintenance of Escherichia coli Laboratory Strains.” Current protocols vol. 1,1 (2021): e20. doi:10.1002/cpz1.20

14. Doyle, M P, and J L Schoeni. “Survival and growth characteristics of Escherichia coli associated with hemorrhagic colitis.” Applied and environmental microbiology vol. 48,4 (1984): 855-6. doi:10.1128/aem.48.4.855-856.1984

15. General Directorate of Public Health. “Laboratuvar Güvenliği Rehberi: Laboratory Safety Guide.” *https://hsgm.saglik.gov.tr/depo/kurumsal/yayinlarimiz/rehberler/UMS-Laboratuvar_GAveliAi_Rehberi-2021_2_versiyon.pdf*, Ministry of Health of the Republic of Turkey, 2021, hsgm.saglik.gov.tr/depo/kurumsal/yayinlarimiz/rehberler/UMS-Laboratuvar_GAveliAi_Rehberi-2021_2_versiyon.pdf

16. "General Directorate of Public Health. "Laboratuvar Güvenliği Rehberi: Laboratory Safety Guide." *https://hsgm.saglik.gov.tr/depo/kurumsal/yayinlarimiz/rehberler/UMS-Laboratuvar_GAveliAi_Rehberi-2021_2_versiyon.pdf*, Ministry of Health of the Republic of Turkey, 2021, hsgm.saglik.gov.tr/depo/kurumsal/yayinlarimiz/rehberler/UMS-Laboratuvar_GAveliAi_Rehberi-2021_2_versiyon.pdf.

17. Lal, Archana, and Naowarat Cheeptham. "Eosin-Methylene Blue Agar Plates Protocol." American Society for Microbiology, 29 Sept. 2007, https://asm.org/ASM/media/Protocol-Images/Eosin-Methylene-Blue-Agar-Plates-Protocol.pdf?ext=.pdf. Accessed 2022.

18. Tarun Madappa, MD. "Escherichia Coli (E Coli) Infections Workup." Laboratory Studies, Imaging Studies, Other Tests, Medscape, 17 Oct. 2021, https://emedicine.medscape.com/article/217485-workup#:~:text=E%20coli%20is%20a%20gram,E%20coli%20strains%20are%20nonpigmented.

19. "CFU: Colony Forming & Calculation." 21 Nov. 2011, http://technologyinscience.blogspot.com/2011/11/cfu-colony-forming-unit-calculation.html. Accessed 2 Dec. 2022.

20. Vuong, Quan & Stathopoulos, Costas & Golding, John & Nguyen, Minh & Roach, Paul. (2011). Optimum conditions for the water extraction of L-theanine from green tea. Journal of separation science. 34. 2468-74. 10.1002/jssc.201100401.

21. "One-Way ANOVA." *One-Way ANOVA - An Introduction to When You Should Run This Test and the Test Hypothesis | Laerd Statistics*, https://statistics.laerd.com/statistical-guides/one-way-anova-statistical-guide.php.

22. "Ethanol (Ethyl Alcohol)." DCCEEW, https://www.dcceew.gov.au/environment/protection/npi/substances/fact-sheets/ethanol-ethyl-alcohol#:~:text=Ethanol%20is%20harmful%20by%20ingestion,tract%20(nose%20and%20throat).

23. Team, Children's Health. "Antibiotics for Acne: How Much Is Too Much?" *Cleveland Clinic*, Cleveland Clinic, 29 Oct. 2021, https://health.clevelandclinic.org/antibiotics-for-acne-how-much-is-too-much/.

Image references:

Figure 1: "Acne Face Map: Causes of Breakouts." Medical News Today, MediLexicon International, https://www.medicalnewstoday.com/articles/325971#acne-locations-and-causes.

Figure 2: "Active Acne Vulgaris: Everything You Need to Know." Premier Clinic, 30 Dec. 2021, https://premier-clinic.com/skin-and-body-problems/active-acne-vulgaris-symptoms-causes/.

APPENDICES

Appendix I: Confirmation letter from Lab. Director, Dr. Ayşegül Akman.

April 28, 2022

To Whom It May Concern,

This letter is written to confirm that the experiment for the biology research project by [redacted] is done under the supervision of Ayşegül Akman.

She has got permission to work with the needed equipment for her experiment to work on stocked Escherichia coli. She has been provided with every lab equipment needed for her experiment and worked by herself under the supervision of microbiologist and laboratory director, Dr. Ayşegül Akman. All work has been done under strict laboratory sterilization conditions.

Yours sincerely,

Ayşegül Akman

Lab. Dr. Microbiologist.

Contact information: aysegulakman@gmail.com

Appendix II: Images taken from phone during the experiment.

Figure-1: Testing tubes after extractions added.

Figure-2: 0.5 mL pipettes

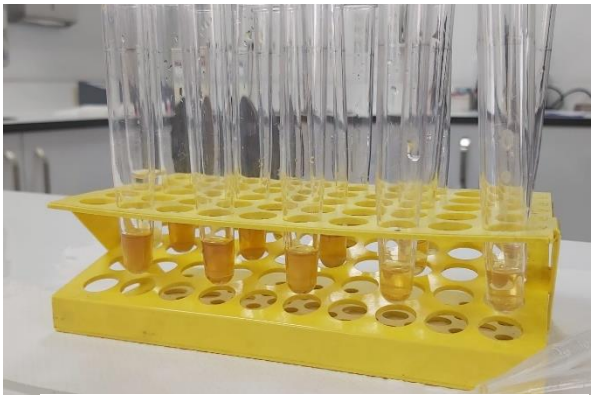


Figure-3: Testing tubes after extractions added.

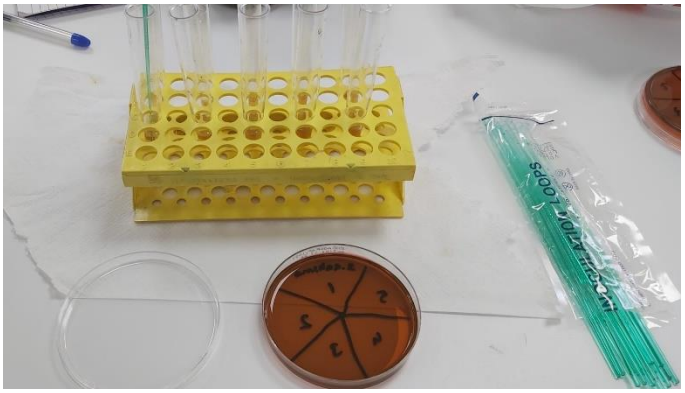


Figure-4: Before inocluation

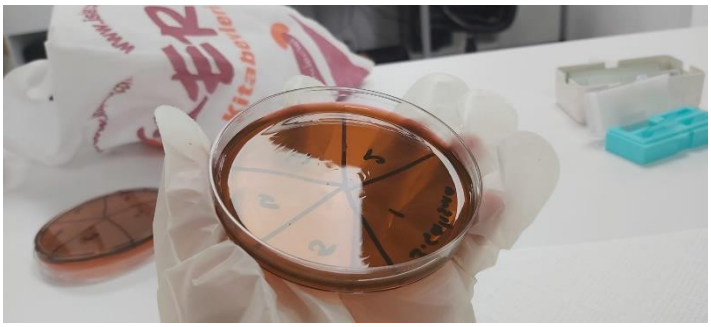


Figure-5: After inoculation

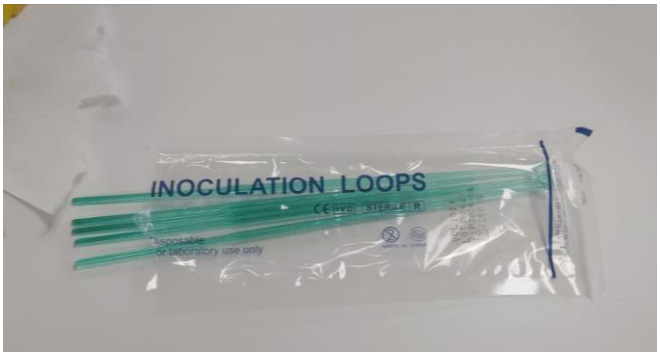


Figure-6: Lab Inoculation loops