A STUDY INTO THE SUITABILITY OF RETINOIDS AS ALTERNATIVE ACNE TREATMENTS IN COMPARISON TO COMMONLY PRESCRIBED ANTIBIOTICAL ACNE TREATMENTS

Extended Essay
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Table of Contents

1. INTRODUCTION

1.1.Research question

Do topical retinoids like adapalene, isotretinoin and tazarotene possess comparable antibacterial effects calculated by the diameter of Zone of Inhibitions against *Escherichia coli* bacteria to commonly prescribed topical antibiotics such as clindamycin?

1.2. Context

Retinoids are the derivates of Vitamin A that include synthetic and natural compounds that have a similar action as that of Vitamin A[1]. Retinoids have different effects on how cells and tissues change in organ culture[2], therefore they are used in cancer chemoprevention in the medical field, specifically in skin cancer where etretinate and acitretin have shown to be effective in inhibiting the development of new dysplastic skin lesions and skin cancers[3]. This epithelial cell homeostasis is demonstrated by the necessitated Vitamin A[4] which is the core substance of all retinoids. In this context, it could be hypothesized that retinoids might greatly impact treating acne vulgaris by stabilizing the epithelial cells on the skin.

[1] Aryal, Arjan, and Sabita Upreti. "A brief review on systemic retinoids." *Int J Pharm Sci Res* 8, no. 9 (2017): 3630-3639.
[2] Lotan, Reuben. "Retinoids in cancer chemoprevention." *The FASEB Journal* 10, no. 9 (1996): 1031-1039.
[3] De Graaf, Y. G. L., S. Euvrard, and J. N. Bouwes Bavinck. "Systemic and topical retinoids in the management of skin cancer in organ transplant recipients." *Dermatologic surgery* 30, no. 4p2 (2004): 656-661.
[4] Dragnev, Konstantin H., James R. Rigas, and Ethan Dmitrovsky. "The retinoids and cancer prevention mechanisms." *The Oncologist* 5, no. 5 (2000): 361-368.

2. BACKGROUND INFORMATION

2.1. Acne vulgaris

Acne vulgaris, simply known as acne, is a chronic inflammatory disease of the pilosebaceous unit resulting from androgen-induced increased sebum production, altered keratinisation, inflammation, and bacterial colonisation of hair follicles on the face, neck, chest, and back.[5] Acne affects about 90% of teenagers[6], and in half of those, the condition persists into adulthood. 1% of males and 5% of women still have lesions at the age of 40[7].

Acne vulgaris is a complex skin condition influenced by both internal and external factors. The primary cause is increased sebum production and abnormal shedding of skin cells, particularly during puberty when hormonal shifts impact gland function.[8] The condition begins with abnormal differentiation of follicular epithelial cells, forming tighter intracellular adhesions and hindering their shedding.[9] The early stage involves the emergence of a microcomedo, obstructing the follicular canal. Increased cohesiveness of corneocytes and hyperkeratosis within the follicular lining lead to the buildup of keratin and sebum. Consequently, a comedone forms over the sebaceous gland duct, and if the pore enlarges, it results in a closed comedone (a firm, elevated, white or yellow papule) or an open comedone (blackhead).[10]

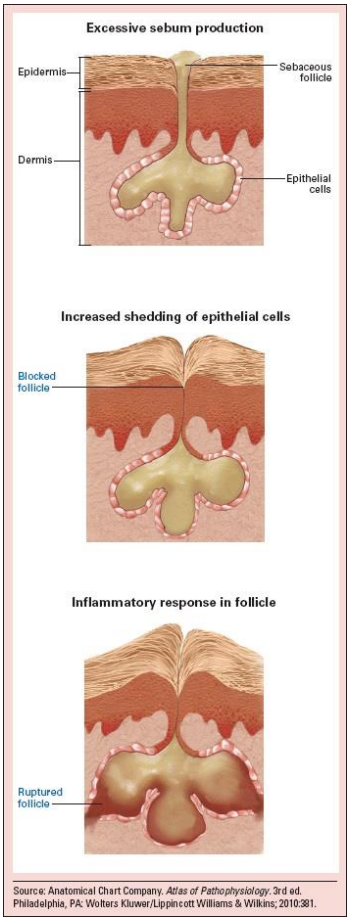

Figure 1: How acne develops

[5] Williams, Hywel C., Robert P. Dellavalle, and Sarah Garner. "Acne vulgaris." *The Lancet* 379, no. 9813 (2012): 361-372.
[6] Department of Internal Medicine, Brigham and Women's Hospital, Boston, MA, USA
[7] Department of Epidemiology, Colorado School of Public Health, University of Colorado Denver, Aurora, CO, USA
[8] Habif TP. *Clinical Dermatology: A Color Guide to Diagnosis and Therapy.* 4th ed. Philadelphia: Mosby; 2004:162-194
[9] Knutsen-Larson, Siri, Annelise L. Dawson, Cory A. Dunnick, and Robert P. Dellavalle. "Acne vulgaris: pathogenesis, treatment, and needs assessment." *Dermatologic Clinics* 30, no. 1 (2012): 99-106.
[10] Well, Danielle. "Acne vulgaris: A review of causes and treatment options." *The Nurse Practitioner* 38, no. 10 (2013): 22-31.

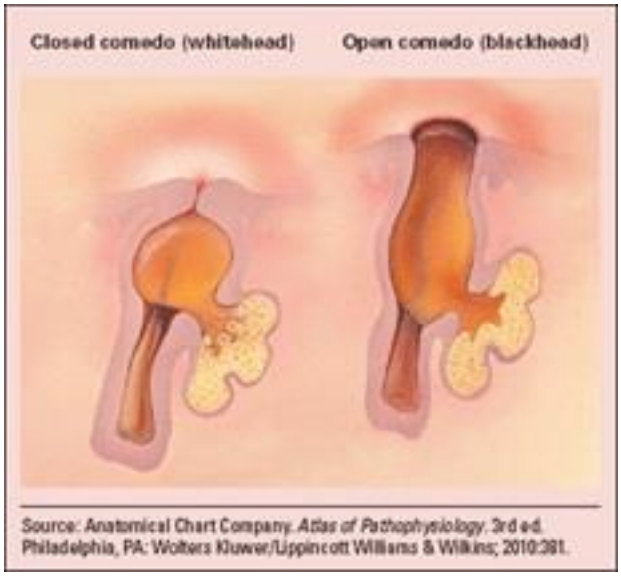

Figure 2: Comedones of acne

2.2. Topical Retinoids

Retinoids are the derivatives of vitamin A that include synthetic and natural substances that have a similar function as that of vitamin A[1].

2.2.1. Adapalene

The synthetic retinoid compound adapalene is a derivative of naphthoic acid. Its mechanism is similar to that of other retinoids and impacts cellular differentiation, keratinization, and the inflammatory process.

In numerous in vitro and in vivo experiments, adapalene has shown moderate to significant anti-inflammatory effects when compared to corticosteroids and nonsteroidal anti-inflammatory medications. Adapalene most frequently causes the previously stated side effects of topical retinoids, such as erythema, scaling, dryness, and burning.[11]

2.2.2. Isotretinoin

The topical formulation isotretinoin is the 13-cis isomer of retinoic acid. The most effective known inhibitor of sebum production is isotretinoin. Isotretinoin is the single most effective medication for the treatment of severe nodulocystic acne and the avoidance of acne scarring

[11] Wolf, J. E. "An update of recent clinical trials examining adapalene and acne." *Journal of the European Academy of Dermatology and Venereology* 15 (2001): 23-29.

due to its multiple mechanisms of action, which include the suppression of sebaceous gland activity, normalization of the pattern of keratinization within the sebaceous gland follicle, and inhibition of inflammation. Together with the well-known brand formulations Roaccutane® and Accutane™ (Roche), several generic oral formulations have recently been developed.[12]

2.2.3. Tazarotene

Tazarotene is a topical retinoid that appears to operate with retinoic acid receptors to produce its effects. It has an anti-inflammatory action and normalizes keratinocyte differentiation and proliferation. It is prescribed to treat psoriasis and acne vulgaris.[13]

2.3. Commonly Prescribed Antibiotics

Dermatology makes use of antibiotics because of their para- and antibacterial effects. Although oral antibiotics have been a key component of therapy for acne for the past 40 years, the condition is not infectious. Tetracyclines in particular, which have anti-inflammatory effects, are effective in treating inflammatory acne.[14]

2.3.1. Clindamycin

Certain bacterial infections, such as those of the lung tissue, the skin, blood, female reproductive systems, and internal organs, are treated with Clindamycin. Clindamycin belongs to the group of drugs known as lincomycin antibiotics. It functions by reducing or halting bacterial growth. Colds, the flu, or other viral infections will not be treated by antibiotics like clindamycin. Antibiotic overuse raises the likelihood that you'll get an infection that is resistant to antibiotic therapy later.[15]

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[12] Ganceviciene, Ruta, and Christos C. Zouboulis. "Isotretinoin: state of the art treatment for acne vulgaris." *JDDG: Journal der Deutschen Dermatologischen Gesellschaft* 8 (2010): S47-S59.
[13] Foster, Rachel H., Rex N. Brogden, and Paul Benfield. "Tazarotene." *Drugs* 55.5 (1998): 705-11.
[14] Farrah, Georgia, and Ernest Tan. "The use of oral antibiotics in treating acne vulgaris: a new approach." *Dermatologic therapy* 29.5 (2016): 377-384.

[15] *Clindamycin: Medlineplus drug information* (no date) *MedlinePlus*. Available at:https://medlineplus.gov/druginfo/meds/a682399.html(Accessed: 15 May 2023).

2.4.Escherichia coli

Excluding *Propionibacterium acnes* from the research due to its opportunistic behaviour and negative host-dependent side effects[16], I had to choose a suitable alternative. After through research, *Escherichia coli (E. coli)* was selected as the model organism since it is a gram-negative bacteria that shares characteristics with the bacteria that cause gram-negative folliculitis, or pustular rashes when acne is treated.[17]. A comparison table between *P. acnes* and *E. coli* was made to support this decision, as shown in Table 1.

Table 1:comparison of P.acnes and E.coli bacteria

Components	*P. acnes*	*E. coli*
Pathogenic	High pathogenicity	Low pathogenicity
Gram stain	Gram-positive	Gram-negative
Reproduction Rate	Approximately 5.1 hours[18]	Every 20 minutes[19]

2.5. Diameter of Zone of Inhibition

This qualitative technique is utilised in both industries, for assessing materials and textiles for microbial resistance, and in medicine, to measure antibiotic resistance. The process involves applying a sterile swab to distribute around a million cells from a single strain onto an agar plate, followed by an incubation period in the presence of the antimicrobial substance. If the bacterial or fungal strain is susceptible, a zone of inhibition appears on the agar plate. However, no noticeable zones emerge if the strain is resistant to the antibacterial agent.[20]

[16]Dréno, B., S. Pécastaings, S. Corvec, S. Veraldi, A. Khammari, and C. Roques. "Cutibacterium Acnes (Propionibacterium Acnes) and Acne Vulgaris: A Brief Look at the Latest Updates." Journal of the European Academy of Dermatology and Venereology 32 (June 2018): 5–14. https://doi.org/10.1111/jdv.15043.

[17] Neubert, Uwe, Thomas Jansen, and Gerd Plewig. "Bacteriologic and immunologic aspects of Gram-negative folliculitis: a study of 46 patients." *International journal of dermatology* 38.4 (1999): 270-274.

[18] Hall, G S et al. "Growth curve for Propionibacterium acnes." *Current eye research* vol. 13,6 (1994): 465-6. doi:10.3109/02713689408999875

[19] 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

[20] Bhargav, H. S., et al. "Measurement of the Zone of Inhibition of an Antibiotic." *2016 IEEE 6th International Conference on Advanced Computing (IACC)*. IEEE, 2016.

3. HYPOTHESIS

Recent studies have indicated that vitamin A may be helpful in skin alternative therapies because it helps the epithelial cells of the skin maintain homeostasis. Retinoids, which are the derivatives of Vitamin A and comprise synthetic and natural substances that have a similar activity to that of Vitamin, may be effective in treating acne vulgaris since vitamin A is beneficial in the treatment of vulgar skin.

Hypothesis 0: After 24 hours of incubation, Clindamycin will have a larger inhibitory effect and a bigger diameter of Zone of Inhibition than retinoids due to its antibacterial properties.

Hypothesis 1: After 24 hours of incubation, the Retinoids will have a larger inhibitory effect and a bigger diameter of Zone of Inhibition than Clindamycin.

4. VARIABLES

4.1. Independent Variable

Substance type is the independent variable used in this experiment. Adapalene (4 g), isotretinoin (4 g), tazarotene (4 g), and clindamycin (4 g) were the medications and concentrations examined in this experiment. Ethanol was utilized as a controlled variable in the experiment because these solutions were made with it as a solvent (25%).

4.2. Dependent Variable

The experiment's dependent variable is the diameter of the zone of inhibitions of *E. coli* after 24 hours of incubation.

4.3. Controlled Variables

Table 2: the controlled variables, their effect on the experiment, their methods for control

Controlled Variables	Effect on the experiment	Method for control
Time of incubation	*E. coli* has a 20-minute doubling time.[21] Given this, the size of the *E. coli* population will grow rapidly as it is incubated for longer, generating a denser bacterial lawn that would make it impossible to compare the substances used in this study fairly.	All Petri dishes were sanitized, isolated, and incubated for 24 hours in a laboratory incubator system.
Incubation temperature	The enzymes of a bacterial species determine what temperature is best for growth as enzyme activity decreases at too low temperatures and increases in temperature may denature enzymes and reduce enzyme activity. Because bacteria have various rates of metabolic activity, they will grow at different rates when incubated at different temperatures, making it impossible to compare them fairly.	30 to 42 degrees Celsius is the optimal temperature range for E. coli growth. The Petri dishes were incubated at 37 degrees Celsius because this is the most ideal temperature.[22]
Strain of *E.coli*	Different *E.coli* strains may vary in their level of resistance to certain chemicals.	All the Petri dishes in the experiment were inoculated with the same strain of *E. coli*, K-12.
The volume of nutrient agar	Since thinner agar layers will have fewer nutrients for the bacteria to grow on, both the rates of diffusion of the antibacterial solutions and the rate of growth of the *E. coli* bacteria can be impacted by the agar layer's thickness.	Throughout the experiment, identical 100mm x 15mm sanitized polystyrene Petri dishes were utilized. A 25 cm^3 volumetric pipette will be used to pour the same volume of nutrient agar solution into each Petri dish, ensuring that the agar is the same thickness throughout all the Petri dishes.
Technical Equipment used	For more accurate findings, the system enables identical mass of bacteria to be placed in testing tubes, solutions, and petri dishes.	All the technical equipment that was used identical.
Sterilization	All used equipment must have as little contamination as feasible throughout the examination. If contamination happens, the experiment's findings could be in danger because new contaminating bacteria and fungi would be introduced into the Petri dishes' sterile environment.	All equipment used was routinely and properly sterilized in accordance with sterilization procedures.

[21] Gibson, Beth, et al. "The distribution of bacterial doubling times in the wild." *Proceedings of the Royal Society B* 285.1880 (2018): 20180789.
[22] Buchanan, R. L., and L. A. Klawitter. "The effect of incubation temperature, initial pH, and sodium chloride on the growth kinetics of Escherichia coli O157: H7." *Food Microbiology* 9.3 (1992): 185-196.

5. MATERIALS

Table 3: materials used in the experiment

Materials	Quantity	Unit (±uncertainty)
Graduated pipette	6	0.5 cm^3 ± 0.005 cm^3
Electronic weighing scale	1	± 0.01 g
E.coli culture	1	-
Beaker	1	1000 mL ± 5mL
Plastic cups with caps	5	150 mL ± 1mL
Polystyrene petri dishes (sterilized)	6	100mm × 15mm
Incubator	1	37 °C ± 0.5 °C
Heat protecting gloves	2	-
Distilled water	-	600 cm^3
Filter paper disks	56	5 mm
97% Ethanol	1	500 cm^3
Nutrient agar powder	1	30 g
Adapalane powder	1	4 g
Tazarotene powder	1	4 g
Isotretinoin powder	1	4 g
Clindamycin powder	1	4 g
Labaratory test tubes	25	-
Labaratory water bath	1	-
Disposable inoculation loops (sterilized)	25	-

6. METHOD DEVELOPMENT AND PLANNING

A scientific microbiology lab is required to study *E. coli* and its response to medications applied. I contacted Prof. Dr. Halil Özdemir, the vice-rector of Ankara University. He gave me permission to carry out my experiment in the university's laboratory in May 2023. (Appendices 1)

First, I needed to decide where to obtain the medication to use for my experiments as the medications required a prescription to buy in pharmacies in Turkey. For this reason, I reached out to the head of the microbiology lab I used, and they obtained the medications for me. Adapalene, Tazarotene, Isotretinoin and Clindamycin were 45 mg, 30 mg, 20 mg, and 75 mg tablets respectively. I decided to use a sample size of 5% concentration for each medication for better accuracy of the study.

After obtaining the medications, I decided to store them in a cabinet in my room at room temperature to keep them away from moisture and limit the exposure to light until the beginning of the study. My aim was to conserve the effectiveness of the drugs as medications are at risk of loss of potency and changes in chemical structure if not stored properly. When the laboratory facilities became available, I would take the drugs to the laboratory, and continue my experiment. At the end of the experiment, I gave the drugs back to the microbiologist who supervised me to safely dispose of them.

Once I had chosen my material, I needed to figure out how to count the bacteria in my samples. The Kirby-Bauer Test, the broth dilution assay, and the culture procedure were the three techniques I could use in this lab. Despite being often employed in microbiological research, the culture method has several drawbacks. This method is labour-intensive and time-consuming, requiring expertise to interpret the data. Additionally, it may underestimate the number of bacteria present, as live cells may not be able to be cultivated using standard

methods.[23] The broth dilution assay also has a number of drawbacks. Rather than offering a proportionate estimate of the antibacterial activity of selected medications, this method qualitatively analyzes the antibacterial effects of specific drugs by observing changes in a solution's turbidity which needs expertise to come to a conclusion. [24] On the contrary, little amounts of medication are required for the Kirby-Bauer test, a technique that provides a proportionate assessment of the antibacterial activity of selected compounds, to produce reliable results.[25]

6.1.1. Experimental Preparations

The initial stage of the preparation process involves creating and transferring the nutrient agar solution into the Petri dishes. This method is based on the University of Utah's approach, utilizing the nutrient agar powder recipe obtained from the laboratory. Table 3 indicates the requirement for six Petri dishes in total. To fulfil this, a total of 150 cm^3 of nutrient agar solution is necessary, as each Petri dish is recommended to receive a 25 cm^3 of the solution.

The second step in the preparation process involves creating a stock plate of *E. coli* bacteria in a sterile environment around a Bunsen burner. Equipment is sterilized using the flame, which also creates an updraft, preventing airborne contamination. The Kirby Bauer method is employed to make the stock plate of *E. coli* bacteria, which was then labelled as such, sealed with Sellotape, and incubated upside-down at 37°C overnight. Antibacterial filter disks are not required for this stock plate.

6.1.2. Plating Bacteria

The retinoid and antibiotic solutions that would impregnate the filter paper disks were made the following day. The antibiotic and retinoids were available as tablets that could be diluted to

[23] Farhoudi, Ayda, et al. "Comparison of Real-time PCR and Cultural Method for Detection of Bacterial Load in Pasteurized Milk." Journal of Food Safety, vol. 39, no. 3, 2019, p. e12624. Crossref, doi:10.1111/jfs.12624.
[24] Dwivedi, Charu, Ishan Pandey, Himanshu Pandey, Pramod W. Ramteke, Avinash C. Pandey, Shanti Bhushan Mishra, and Sandip Patil. "Electrospun Nanofibrous Scaffold as a Potential Carrier of Antimicrobial Therapeutics for Diabetic Wound Healing and Tissue Regeneration." In Nano- and Microscale Drug Delivery Systems , 147–64. Elsevier, 2017. https://doi.org/10.1016/B978-0-323-52727-9.00009-1.
[25] Antimicrobial Susceptibility Testing EUCAST Disk Diffusion Method. EUCAST, January 2019.

create solutions with 4% concentrations. Since none of the four drugs were soluble in water, ethanol was employed to reconstitute them. The formula used to create the retinoid and antibiotic solutions is shown in Table 4:

Table 4: the amount of drugs and ethanol used

Solution Name	Solution Description
Adapalene 5%	45 mg of adapalene mixed with 1.125 cm^3 of ethanol
Tazarotene 5%	30 mg of tazarotene mixed with 0.75 cm^3 of ethanol
Isotretinoin 5%	20 mg of isotretinoin mixed with 0.5 cm^3 of ethanol
Clindamycin 5%	75 mg of clindamycin mixed with 1.87 cm^3 of ethanol

Subsequently, 56 filter paper disks with a diameter of 6mm were punctured, placed in McCartney bottles, and subjected to sterilization in a pressure cooker. Following this, the stock plate was taken out of the incubator, and the nutrient agar Petri dishes were retrieved from the refrigerator. Setting up a Bunsen burner, sanitizing the workstation with ethanol, and organizing and labelling the Petri dishes in alignment with the plating strategy followed.

The Kirby Bauer method was used to inoculate each Petri dish with bacteria from the *E.coli* stock plate and apply the appropriate filter paper disks afterwards. The following 48 hours were spent incubating them upside-down at 37°C.

6.1.3. Collecting Data

After 24 hours, the diameter of the ZoI encircling the filter paper disk was measured with a Vernier calliper.

Procedure

Obtaining materials:

- Obtain adapalene, tazarotene, isotretinoin and clindamycin tablets from a pharmacy
- Keep them in a cabinet away from light, high temperature and moisture

Making Agar Solution:

- Measure 150 cm^3 of distilled water in a 250 cm^3 graduated cylinder, pour it into a 250 cm^3 conical flask, and set it on the magnetic stirrer.
- Proceed to weigh 17.8 grams of the nutrient agar powder using an electronic scale. Slowly add the powder to the conical flask, letting it dissolve entirely.
- Seal the mouth of the conical flask with a piece of cotton to allow the steam to pass.
- Sterilize the conical flask for 30 minutes at 15 psi in the pressure cooker.
- Use a 25 cm^3 volumetric pipette to pick up 25 cm^3 sterilized nutrient agar and pour it into each individual Petri dish.
- Wait 15 minutes for the agar to solidify.
- Wrap the Petri dishes and keep them inverted in a refrigerator at 4 °C.

Making A Stock Plate

- Use ethanol to disinfect the workstation, then set up a Bunsen burner.
- In one motion, inoculate the *E. coli* stock plate with bacteria with a sterile inoculation loop. Then place the bacteria on the nutrient agar.
- Form a bacterial lawn by spreading the bacteria across the surface of nutrient agar.
- Sterilize forceps and use them to pick up a filter paper disk and dip it into the required solution.
- Place the filter paper disk on the surface of the nutrient agar and tap it gently so that it lies flat.
- Close and seal the petri dishes with sellotape.
- Incubate the petri dishes upside down at 37 °C.

Plating Bacteria

- After 24 hours the solutions of drugs and ethanol were created (Table 4)
- 56 6mm filter paper were punched out using a hole punch

- Take the stock plate out of the incubator and the nutrient agar filled Petri dishes out of the refrigerator.
- Inoculate each Petri dish using bacteria from the *E. coli* stock plate.
- Incubate them for 24 hours at 37 °C.
- Measure the diameter of the ZoI surrounding the filter paper disk with Vernier calliper.
- Repeat the steps 8 times for each trial.

7. DATA COLLECTION AND PROCESSING

I completed eight trials—a total of repeat trials—for each type of medication. For every sample, the ZoI was calculated while varying each medication. As a result, the findings of these 8 trials, each using different kinds of medicine (for a total of 28 records), appear adequate to monitor the effects of different types of drugs on the ZoI diameter.

A Vernier Calliper was used to calculate the diameter of circular ZoI. However, the ZoI that were not entirely circular were calculated by:

$$\frac{maximum\ diameter + minimum\ diameter}{2}$$

Example Calculation for Adapalene:

$$\frac{15.37+13.83}{2} = 14.60$$

Table 5: Raw Data of trials, diameters of zone of inhibition on nutrient agar among different drugs with same 5% concentrations (Adapalene, Tazarotene, Isotretinoin and Clindamycin)

Trials	*Diameter of ZoI* mm ± 0.05				Incubation time (hour)	agar volume (cm^3 ±0.5)	Incubation temperature (°C ±0.5°C)
	Adapalene 5%	Tazarotene 5%	Isotretinoin 5%	Clindamycin 5%			
1	14.60	12.55	18.53	7.48	24	25.0	37
2	14.44	12.42	17.49	8.65	24	25.0	37
3	14.87	11.70	18.55	8.73	24	25.0	37
4	15.45	11.63	19.25	9.14	24	25.0	37
5	13.80	13.10	18.79	8.58	24	25.0	37
6	15.25	12.70	19.86	8.75	24	25.0	37
7	14.75	11.67	19.74	8.70	24	25.0	37
8	14.58	13.24	18.93	9.40	24	25.0	37

7.1.Statistical Calculations

1) Mean of the diameters of ZoI in the same group

$$\mu = \frac{\sum_{i=1}^{n} x_i}{n}$$

where

n is the number of trials in a certain data group

x is the diameter of ZoI in a single trial

Example (Adapalene): $\frac{14.60 + 14.44 + 14.87 + 15.45 + 13.80 + 15.25 + 14.75 + 14.58}{8} = 14.72$

2) Standard deviation of the diameter of ZoI in the same data group

$$\sigma = \sqrt{\frac{\sum (x_i - \mu)^2}{N}}$$

where

x is the diameter of ZoI in a single trial

μ is the mean number of diameters of ZoI of a particular drug

n is the number of trials in a data group

Example (Adapalene):

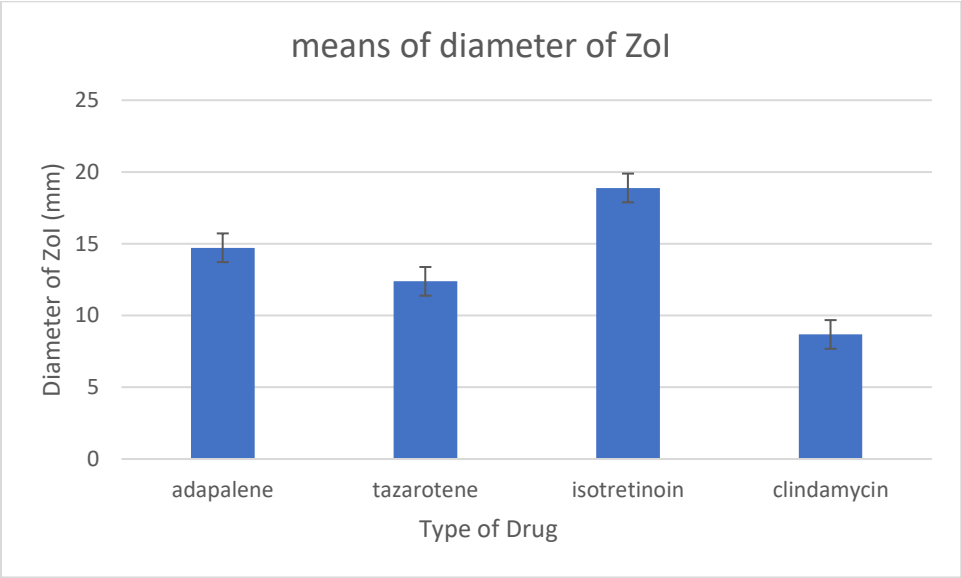
$$\sqrt{\frac{(14.60-14.72)^2+(14.44-14.72)^2+(14.87-14.72)^2+(15.45-14.72)^2+(13.80-14.72)^2+(15.25-14.72)^2+(14.75-14.72)^2+(714.58-14.72)^2}{8}}$$

$$\cong 0.47$$

Table 6: Mean and Standard deviation of each type of medication

Drug name	Adapalene	Tazarotene	Isotretinoin	Clindamycin
mean number of diameters of ZoI of a particular drug	14.72	12.38	18.89	8.68
Standard deviation	0.47	0.60	0.71	0.52

7.2. Graph



Graph 1:Processed bar graph of means of diameter of ZoI of Adapalene, Tazarotene, Isotretinoin, and Clindamycin. Error bars are added by standard deviation.

7.3. ANOVA

ANOVA, or analysis of variance, is a statistical technique that divides observed variance data into distinct components for use in further testing. A one-way ANOVA is utilized to determine the correlation between the independent and dependent variables when analyzing data from

three or more groups. A null hypothesis pertaining to my experiment's hypothesis must be established before doing ANOVA tests.

H0: There is no statistical difference between retinoids or commonly prescribed antibiotics on ZoI of *E.Coli*

H1: There is a statistical difference between retinoids or commonly prescribed antibiotics on ZoI of *E.Coli*

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
adapalene	8	117,74	14,7175	0,25656429
tazarotene	8	99,01	12,37625	0,4171125
isotretinoin	8	151,14	18,8925	0,57282143
clindamycin	8	69,43	8,67875	0,31266964

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	439,664575	3	146,554858	375,982246	1,0257E-22	2,94668527
Within Groups	10,914175	28	0,38979196			
Total	450,57875	31				

Table 7: Results of ANOVA Single Factor

Based on the outcomes of the ANOVA: Single Factor test, the P-value is calculated as 1,0257E-22 (Table 5). The sample is inconsistent with H0 (the null hypothesis), which may be rejected for the full data set, and the variations between the means are statistically significant because the P-value is less than the significance threshold (0.05). This makes the results' validity stronger. Thus, H1 is accepted.

7.4. Tukey's Range Test

The ANOVA findings that are shown do not specify which specific differences between the trial pairings' means are significant. Given that I have the same number of trials in each pair, I utilize Tukey's range tests to examine the differences between the means of the group pairs.

Tukey HSD results

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
A vs B	10.6066	0.0010053	** p<0.01
A vs C	27.3574	0.0010053	** p<0.01
A vs D	18.9141	0.0010053	** p<0.01
B vs C	16.7508	0.0010053	** p<0.01
B vs D	29.5207	0.0010053	** p<0.01
C vs D	46.2715	0.0010053	** p<0.01

Table 8: Results of Tukey's Range Test where Groups A, B, C and D are Adapalene, Tazarotene, Isotretinoin and Clindamycin respectively.

All p-values of the groups are below the critical value which is 0.05. This means that every pair of groups differs from each other significantly.

8. ANALYSIS

The zone of inhibition diameters was statistically different between all data groups (Table 6, Graph 1). The smallest ZoI was produced by the commonly prescribed antibiotic "Clindamycin" and the largest ZoI was produced by the retinoid "Isotretinoin". The ANOVA: Single Factor test showed a statistical connection between the data sets which made me accept my H1. By utilising the Tukey's Range Test, it could be analyzed which data groups were more different than the other ones. From Tukey's Test, it could be seen that Clindamycin and Isotretinoin had the biggest difference between the whole data set. As the aim of this study was to compare the antibacterial effects of retinoids and the commonly prescribed antibiotic

Clindamycin, the difference between the diameters of zone of inhibitions and the statistical tests employed led me to accept my H1.

The inhibitory capacities of different types of retinoids were also studied in this experiment. The difference between their inhibitory capacities can be seen in Graph 1 clearly. Among the retinoids, Isotretinoin had the most followed by Adapalene and lastly, Tazarotene. This result showed that different types of retinoids did have different antibacterial activity which proved that it was not just about the type of medication that affected acne growth. Among all the medications antibiotics is the least effective.

The standard deviations of the data sets were relatively small with the highest one being 0.71 and the lowest one being 0.47 belonging respectively to Isotretinoin and Adapalene. A small standard deviation indicates that the findings are near the mean; a big standard deviation indicates that the data are more dispersed.[26] The standard deviations being small means that the experiment was precise which enforces the fact that my H1 was right. The fluctuations between the data groups being small might have happened because the agar solutions were prepared and incubated at the same time in the same incubator which reduces systematic error in the experimental preparations.

9. EVALUATION

This study has a few limitations. The drugs used in the experiment had varying concentrations because they were supplied in standard-sized doses. Due to random errors during the hand-dilution process of the solutions to 5% each, this variation may have had an impact on the number of bacteria. To improve the validity of the results, it is possible to compensate for this variable by standardizing the medication concentrations in subsequent investigations.

To achieve a more comprehensive review of the outcomes, the method can also be improved. One way to do this would be to measure the ZoI more regularly and incubate the plates for a

[26] Anonymous (2021). Measures of the Spread of Data [Online]. https://courses.lumenlearning.com/introstats1/chapter/measures-of-the-spread-of-data/. Accessed on 15 Nov. 2023.

longer amount of time. This would draw attention to the variations in ZoI size over time and may reveal compounds that lose their antibacterial efficacy after a 24-hour incubation period. Another way to improve the methodology of the experiment is to use more concentrations for each substance. This would give a more comprehensive analysis of the inhibitory effects of the drugs chosen.

This was a highly standardized experiment despite these drawbacks. Since most of the factors were considered, the experiment can be repeated with identical circumstances, allowing the dependability of the findings to be confirmed. The study's utilization of a hospital's microbiology lab, which employs the extremely sensitive Kirby Bauer method, was another strength.

10. CONCLUSION

In conclusion, the experiment and analysis justified the research question: Do topical retinoids like adapalene, isotretinoin and tazarotene possess comparable antibacterial effects against *Escherichia coli* bacteria to commonly prescribed topical antibiotics such as clindamycin?

The inhibitory capacities of topical retinoids support the hypothesis and can be considered as an alternative way to treat Acne vulgaris at a time when the resistance of bacteria to antibiotics has increased. However, topical retinoids should not be used without the permission of a professional as they are regarded as toxic when not used properly or cause allergy to the person using them.[27] If a patient shows signs of allergy to different kinds of retinoids, they might be put on antibiotics instead by a dermatologist. A medication's ability to decrease antibacterial activity is not the only thing considered when prescribing it to a patient.

For a more comprehensive study, different types of commonly prescribed antibiotics might be added to the methodology. The new research question might be: Do topical retinoids like adapalene, isotretinoin and tazarotene possess comparable antibacterial effects against

[27] Sato, Yuji, et al. "Dietary carotenoids inhibit oral sensitization and the development of food allergy." *Journal of Agricultural and Food Chemistry* 58.12 (2010): 7180-7186.

Escherichia coli to commonly prescribed topical antibiotics such as clindamycin, erythromycin, and doxycycline?

11. REFERENCES

1. Aryal, Arjan, and Sabita Upreti. "A brief review on systemic retinoids." *Int J Pharm Sci Res* 8, no. 9 (2017): 3630-3639.
2. Lotan, Reuben. "Retinoids in cancer chemoprevention." *The FASEB Journal* 10, no. 9 (1996): 1031-1039.
3. De Graaf, Y. G. L., S. Euvrard, and J. N. Bouwes Bavinck. "Systemic and topical retinoids in the management of skin cancer in organ transplant recipients." *Dermatologic surgery* 30, no. 4p2 (2004): 656-661.
4. Dragnev, Konstantin H., James R. Rigas, and Ethan Dmitrovsky. "The retinoids and cancer prevention mechanisms." *The Oncologist* 5, no. 5 (2000): 361-368.
5. Williams, Hywel C., Robert P. Dellavalle, and Sarah Garner. "Acne vulgaris." *The Lancet* 379, no. 9813 (2012): 361-372.
6. Department of Internal Medicine, Brigham and Women's Hospital, Boston, MA, USA
7. Department of Epidemiology, Colorado School of Public Health, University of Colorado Denver, Aurora, CO, USA
8. Habif TP. *Clinical Dermatology: A Color Guide to Diagnosis and Therapy*. 4th ed. Philadelphia: Mosby; 2004:162-194
9. Knutsen-Larson, Siri, Annelise L. Dawson, Cory A. Dunnick, and Robert P. Dellavalle. "Acne vulgaris: pathogenesis, treatment, and needs assessment." *Dermatologic Clinics* 30, no. 1 (2012): 99-106.
10. Well, Danielle. "Acne vulgaris: A review of causes and treatment options." *The Nurse Practitioner* 38, no. 10 (2013): 22-31.
11. Wolf, J. E. "An update of recent clinical trials examining adapalene and acne." *Journal of the European Academy of Dermatology and Venereology* 15 (2001): 23-29.

12. Ganceviciene, Ruta, and Christos C. Zouboulis. "Isotretinoin: state of the art treatment for acne vulgaris." *JDDG: Journal der Deutschen Dermatologischen Gesellschaft* 8 (2010): S47-S59.
13. Foster, Rachel H., Rex N. Brogden, and Paul Benfield. "Tazarotene." *Drugs* 55.5 (1998): 705-11.
14. Farrah, Georgia, and Ernest Tan. "The use of oral antibiotics in treating acne vulgaris: a new approach." *Dermatologic therapy* 29.5 (2016): 377-384.
15. *Clindamycin: Medlineplus drug information* (no date) *MedlinePlus*. Available at: https://medlineplus.gov/druginfo/meds/a682399.html (Accessed: 15 May 2023).
16. Dréno, B., S. Pécastaings, S. Corvec, S. Veraldi, A. Khammari, and C. Roques. "Cutibacterium Acnes (Propionibacterium Acnes) and Acne Vulgaris: A Brief Look at the Latest Updates." Journal of the European Academy of Dermatology and Venereology 32 (June 2018): 5–14. https://doi.org/10.1111/jdv.15043.
17. Neubert, Uwe, Thomas Jansen, and Gerd Plewig. "Bacteriologic and immunologic aspects of Gram-negative folliculitis: a study of 46 patients." *International journal of dermatology* 38.4 (1999): 270-274.
18. Hall, G S et al. "Growth curve for Propionibacterium acnes." *Current eye research* vol. 13,6 (1994): 465-6. doi:10.3109/02713689408999875
19. 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
20. Bhargav, H. S., et al. "Measurement of the Zone of Inhibition of an Antibiotic." *2016 IEEE 6th International Conference on Advanced Computing (IACC)*. IEEE, 2016.
21. Gibson, Beth, et al. "The distribution of bacterial doubling times in the wild." *Proceedings of the Royal Society B* 285.1880 (2018): 20180789.

22. Buchanan, R. L., and L. A. Klawitter. "The effect of incubation temperature, initial pH, and sodium chloride on the growth kinetics of Escherichia coli O157: H7." *Food Microbiology* 9.3 (1992): 185-196.
23. Farhoudi, Ayda, et al. “Comparison of Real-time PCR and Cultural Method for Detection of Bacterial Load in Pasteurized Milk.” Journal of Food Safety, vol. 39, no. 3, 2019, p. e12624. Crossref, doi:10.1111/jfs.12624.
24. Dwivedi, Charu, Ishan Pandey, Himanshu Pandey, Pramod W. Ramteke, Avinash C. Pandey, Shanti Bhushan Mishra, and Sandip Patil. “Electrospun Nanofibrous Scaffold as a Potential Carrier of Antimicrobial Therapeutics for Diabetic Wound Healing and Tissue Regeneration.” In Nano- and Microscale Drug Delivery Systems , 147–64. Elsevier, 2017. https://doi.org/10.1016/B978-0-323-52727-9.00009-1.
25. Antimicrobial Susceptibility Testing EUCAST Disk Diffusion Method. EUCAST, January 2019.
26. “Making Agar Plates.” 2015. Accessed August 4, 2019. https://teach.genetics.utah.edu/content/microbiology/plates/.
27. Anonymous (2021). Measures of the Spread of Data [Online]. https://courses.lumenlearning.com/introstats1/chapter/measures-of-the-spread-of-data/. Accessed on 15 Nov. 2023.
28. Sato, Yuji, et al. "Dietary carotenoids inhibit oral sensitization and the development of food allergy." *Journal of Agricultural and Food Chemistry* 58.12 (2010): 7180-7186

12. APPENDICES

Appendix 1: Confirmation letter from vice rector Halil Özdemir

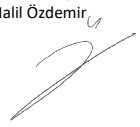
May 21, 2023

To Whom It May Concern,

This letter is written to confirm that the experiment for the biology extended essay by [redacted] is done under the supervision of Halil Özdemir.

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 Dr. Halil Özdemir. All work has been done under strict laboratory sterilization conditions.

Yours sincerely,
Dr. Halil Özdemir



Contact information: doktorhalil@gmail.com