A Novel Approach: Diagnosing, Quantifying and Tracking the Progression of Inflammation-Causing Skin Diseases Using Handheld Optical Reflectance Spectrophotometry

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Objective

The objective of this experiment was to determine if a handheld optical spectrophotometer can be used to accurately provide reflectance indexes and measurements to distinguish between different skin tones and also distinguish between different skin conditions and normal versus affected areas based on the reflectance measurements.

Background Research

Millions of people are affected by many skin conditions that are caused by either immune system problems or irritants that lead to the development of inflammation or plaques on the epidermis. These diseases, such as psoriasis or eczema, do not have readily-available cures and are usually chronic. Both the diagnosis and progression of these skin conditions are typically invasive and involve a skin biopsy. This can cause sensitivity and irritation to the biopsy site and if repeated frequently if the condition worsens rapidly, can be quite damaging to the skin and other affected areas. Scientists have been investigating the use of different types of spectroscopy to help make the process of diagnosing and monitoring these skin conditions non-invasive. Another common way to diagnose skin conditions is visually by a dermatologist using different ways to score a condition. However, this is usually a highly subjective process and the likeliness of misdiagnosing a condition or being unsure of a diagnosis is highly possible. Arizona State University's SciHub team created a portable handheld optical reflectance spectrophotometer that can be used in versatile ways, leading to the question: can a handheld optical spectrophotometer be used to diagnose, quantify, and track the progression of skin conditions that cause inflammation, irritation, plaques, or erythema on the skin and provide accurate data that correlates with histological analyses? This question is significant to people who believe they may be affected by these skin conditions, suffer from them, and dermatologists can use this tool as an additional way to confirm diagnoses and prognoses.

Experimental Design

Independent Variables: The independent variables in this experiment were the different wavelengths of light (450 nm, 500 nm, 530 nm, 613 nm, 634 nm, 665 nm, 880 nm, and 940 nm) and the skin tones of the participants.

Dependent Variables: The dependent variables in this experiment were the reflectance measurements and their ability to help differentiate between different skin types and conditions.

Standardized Variables: The constants are the amount of time the spectrophotometer emits light, the time of day, the positioning of the spectrophotometer on the plaque/inflammation/skin, and the number of readings per each area of affected/unaffected skin.

Control Group: The control in this experiment was the unaffected skin areas (no signs of any skin disease).

Hypothesis 1: A handheld optical reflectance spectrometer that emits light at different wavelengths can be used to produce reflective indexes and distinguish between patients with different skin tones and will be able to differentiate between different skin tones because of the differing melanin concentrations in the skin which causes different amounts of light to be absorbed and reflected by the epidermis (Ou-Yang et al).

Hypothesis 2: A handheld optical reflectance spectrometer can be used to produce reflective indexes and distinguish between different skin tones and can also be used to identify different skin conditions based on the differences in the reflectance measurements (Ou-Yang et al).

Replication/Sample Size: Each level was repeated 5 times to get a total of 5 readings per each wavelength.

Materials and Methods:

Materials:

- SciHub Spectrophotometer
- Micro USB cable
- Arduino Computer Program
- Antibacterial wipes
- Laptop
- Participants who are clinically diagnosed with psoriasis, contact dermatitis, or eczema
- Participants with different skin tones based on the Fitzpatrick scale
    - Patient 1 - type III
    - Patient 2 - type IV
    - Patient 3 - type II
    - Patient 4 - type V
    - Patient 5 - type III
    - Patient 6 - type III

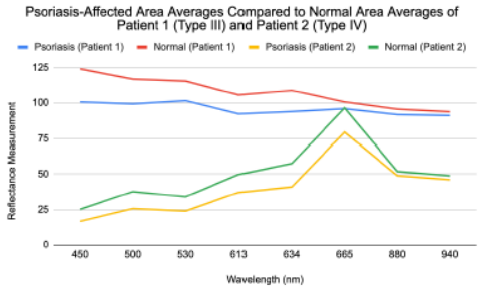
Procedures:

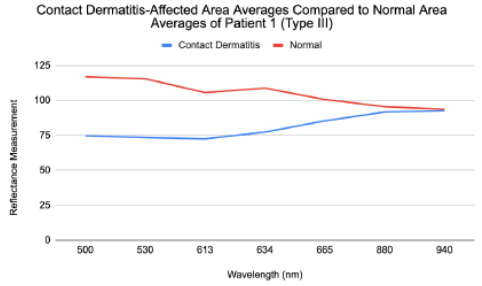
1. Gather and purchase all materials.
2. Find participants who have been clinically diagnosed with psoriasis, contact dermatitis, or eczema, as well as participants with different skin tones according to the Fitzpatrick scale.
3. Connect the spectrophotometer to a laptop using the micro USB cable and the Arduino computer program. Follow the instructions given with the spectrophotometer for this setup.

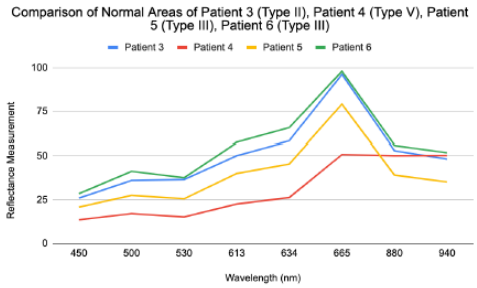
4. Find and determine four affected areas on the participant's skin that are affected by the skin condition. Clean the areas with antibacterial wipes or have the participant wash their hands before collecting data.
5. Place the spectrophotometer flush against the first affected area and use the BlueFruit app and spectrophotometer to determine the reflectance measurements which will be displayed on the screen monitor on Arduino.
6. Continue to have the participant hold the spectrophotometer in the same position against the affected area and collect measurements four more times.
7. Repeat steps 5 and 6 for the remaining three affected areas.
8. Place the spectrophotometer flush against a sample of skin that is completely unaffected by the skin condition, such as a patch of skin on the palm or wrist, and determine the reflectance measurements for the control group (unaffected skin).
9. Repeat step 8 four more times to gather a total of 5 sets of data for the unaffected skin area.
10. Average all the data for each wavelength and graph the data points to compare the reflectance measurements of affected versus unaffected skin.
11. When finished with data collection, clean the spectrophotometer using the antibacterial wipes.
12. For comparison of different skin tones of patients who are unaffected with any skin condition, complete step 6, five times and calculate the average reflectance measurements.
13. Repeat steps 5 through 11 for all remaining participants who are affected with the skin condition(s) and step 12 for unaffected patients.

Statistical Methods: At the conclusion of the experiment, the average reflectance measurements for each wavelength were calculated by adding all the measurements together and dividing by the number of trials.

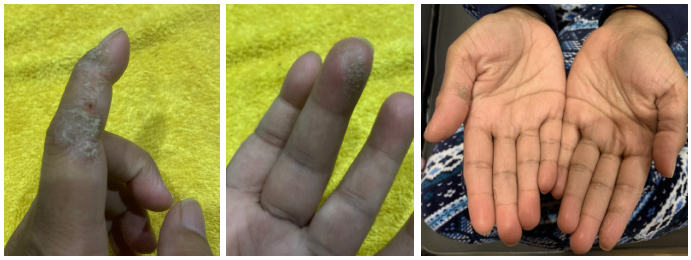
Results







Patient 1 Pictures



Patient 2 Pictures

Data Analysis

Graph 1 displays the reflectance measurements from patient 1 and patient 2, both who have been diagnosed with psoriasis. Graph 1 depicts the average reflectance measurements for the psoriasis-affected areas of patient 1 at different wavelengths, each affected area being labeled as a separate line. In general, all the affected areas have similar reflectance measurements, ranging from approximately 100.6 reflectance at 450 nanometers to approximately 91.1 reflectance at 940 nanometers. As the wavelength increases, the reflectance of the psoriasis-affected areas decreases. On the other hand, when the psoriasis-affected area reflectance measurements are compared to the normal skin's reflectance measurements, the normal area has higher average reflectance measurements for all wavelengths, but a most significant difference of 23.49 at 450 nanometers. Graph 1 also displays the reflectance measurements from patient 2. Similarly to patient 1, as the wavelength increases, the reflectance of the psoriasis-affected areas decreases. The reflectance measurements for the normal area is also higher than the affected areas. In general, the reflectance measurement at 450 nanometers is approximately 16.7 and approximately 46.121 at 940 nanometers. The most significant difference is at 665 nanometers where the normal area has a reflectance measurement higher than the affected area by 16.94. The normal area's average reflectance measurements are higher than the psoriasis-affected areas for both patients, with the exception of the 880-940 nanometer region,

where the psoriasis-affected areas' averages grow closer to the normal area averages. In the 450 nm region, there is a difference of approximately 23.49 for patient 1 and approximately 8.45 for patient 2. Graph 2 also indicates this for contact dermatitis; the difference between the affected areas and normal areas is most significant in the 450-665 nanometer region. In the 450 nm region, there is a difference of approximately 45.194. Whereas, in the 940 nm region, there is a difference of approximately 1.05. Additionally, patient 1 and patient 2 have different reflectance measurements in the normal skin areas as well. In the 450 nanometer region, there is a difference of approximately 98.95 and in the 940 nanometer region, there is a difference of approximately 44.8. However, in the 665 nanometer region, the difference between both areas is smaller, approximately only 4.19. Overall, this means that in both skin conditions, more light is being reflected in the normal skin areas compared to the affected skin areas, however, the difference is more noticeable in the contact dermatitis-affected areas. Graph 3 shows the different reflectance measurements between patients 3, 4, 5 and 6, who all have different skin types which are marked on the graph. Patient 4 had the darkest skin tone (type V), therefore also having the lowest reflectance measurements. As the skin tone grew lighter, the reflectance measurements increased, with patient 6 (type II) reflecting the most light at 665 nanometers. For instance, at 665 nanometers, there was a difference of 47.126 between patient 4 and patient 6. This shows that overall, patients with darker skin tones (type V-VI) generally had lower reflectance measurements in comparison to patients with lighter skin tones (type I-III).

Conclusion

The purpose of this experiment was to determine if an optical spectrophotometer can be used to accurately provide reflectance indexes and measurements to distinguish between different skin tones and also distinguish between different skin conditions based on the reflectance measurements. There were two hypotheses in this experiment: 1) A handheld optical reflectance spectrometer that emits light at different wavelengths can be used to produce reflective indexes and distinguish between patients with different skin tones and will be able to differentiate between different skin tones because of the differing melanin concentrations in the skin which causes different amounts of light to be absorbed and reflected by the epidermis, and 2) A handheld optical reflectance spectrometer can be used to produce reflective indexes and distinguish between different skin tones and can also be used to identify different skin conditions based on the differences in the reflectance measurements (Ou-Yang et al). Overall, the data collected supports both hypotheses. For hypothesis 1, as shown in Graph 3, the handheld optical reflectance spectrophotometer was able to differentiate between different skin types (patient 3 -type II, patient 4 - type V, patient 5 - type III, patient 6 - type III). This can be seen because of the difference reflectance measurements, meaning that patient 6, with the lowest amounts of melanin, overall, had higher reflectance measurements in comparison to patient 4, with higher amounts of melanin, but lower reflectance measurements. A significant difference can be observed at the 665 nanometer region for all skin tones. This data is supported by previous research that people with greater amounts of melanin tend to absorb more light and reflect less light (Stanford). This supports hypothesis 1 - that the optical reflectance spectrophotometer will be able to produce different reflective indexes for people with different skin tones. The most significant difference can be seen at 655 nanometers. For hypothesis 2, graphs 1 and 2 depict the differences between the affected versus normal areas for both patients. The reflectance measurements for the affected areas of both patients were lower than the reflectance measurements of the normal areas. In both psoriasis and contact dermatitis, the reflectance measurements of the affected areas were lower than the normal areas. Therefore, this experiment suggests that a handheld optical reflectance spectrophotometer can be used to both distinguish between skin tones and also quantify and track the progression of skin conditions based on the reflectance measurements. Some possible errors were slightly inaccurate measurements since the handheld spectrophotometer is not as accurate as larger, professional spectrophotometers as well as potential movement of the spectrophotometer in between readings. In the future, measurements should be taken from more patients who are affected by these skin conditions in order to provide a greater sample size. Overall, the findings from this study are supported by findings from previous studies; different methods of spectroscopy have potential to be used to provide a noninvasive way to dynamically monitor skin conditions and the changes they cause to melanin production and the microvasculature.

Literature Cited

Ou-Yang, Hao, et al. "Spectral Responses of Melanin to Ultraviolet A Irradiation." Journal of Investigative Dermatology, vol. 122, no. 2, 2004, pp. 492–496.,doi:10.1046/j.0022-202x.2004.22247.x.

(n.d.). Retrieved from https://genetics.thetech.org/ask-a-geneticist/does-ability-tanburn-have-something-do-gene tics#:~:text=Melanin effectively absorbs light., the harmful effects of sunlight.

A Novel Approach to Healing Burn Wound Infections using Flavonoids

Michelle Sheikh

Abstract

The purpose of this experiment is to find a novel way to treat burn wound infections instead of using the standard antibiotics. 'Flavonoids' are naturally derived secondary metabolites which have the potential to treat burn wound infections just as effectively as conventional antibiotics. The significance of this experiment lies in the fact that over 40% of burn wound deaths are due to infection. Antibiotic resistance has been causing serious complications for the healing of burn wounds. There has been substantial research exploring the use of an alternative to antibiotics such as flavonoids. The flavonoids, Quercetin, and any Quercetin combined compound, when compared to Rutin and Hesperidin alone, will be the most effective antibiotic alternative against *E. coli* and *S. epidermidis*, the two most common bacterial infections found in burn wound infections. The five hydroxyl groups present in Quercetin chemical structure enable it to possess enhanced antibacterial activity (Han, 2018). Therefore, Quercetin will have the highest success rate as an antibiotic alternative because of its strong antibacterial nature. Quercetin has also shown significant antibacterial activity against methicillin-resistant *S. aureus* (Geoghegan & Wang, 2015).

The effect of different flavonoids on wound infecting bacteria *E. coli* and *S. epidermidis* was determined by flooding Petri dishes with *E. coli* and *S. epidermidis* and placing an antibiotic disk made of either an isolated flavonoid or combinations of flavonoids.The results collected during this experiment concluded that the combination of all their flavonoids (Quercetin, Rutin, and Hesperidin) was the most effective at inhibiting bacterial growth at the 50% concentration for *E. coli* and the 25% concentration for *S. epidermidis*. Therefore the hypothesis was disproved because the QHR combination may have worked the best due to synergy occurring between the flavonoids. A study done by the University of Science and Technology of China tested synergistic action between Rutin, Morin, and Quercetin found that synergy occurs between the flavonoids resulting in a greater MIC (minimum inhibitory concentration). Possible errors include contamination due to the use of ziploc bags and the use of distilled water to create the weight-based concentrations instead of ethanol. Future Research regarding the use of flavonoids could be evaluating the possible synergistic relationship by combining flavonoids and synthetic antibiotics as another potential solution to counter antibiotic resistance. Additionally, the antiviral activities of flavonoids can be investigated for use against global health threats such as the Covid-19 virus.

Question Tested:
How do different types of flavonoids such as Quercetin, Rutin, and Hesperidin at varying concentrations inhibit burn wound infecting bacteria such as *Escherichia coli* and *Staphylococcus Epidermidis*?

Independent Variable:
The manipulated variable was the type of flavonoid/ antibiotic used. The natural antibiotic used was a diluted solution of Quercetin, Rutin, Hesperidin or mixtures of these flavonoids which was measured in grams. The synthetic antibiotic used was a diluted solution of Amoxicillin which was measured in milliliters.

Dependent Variable:
The responding variable was the size of the Zone of Inhibition (mm) present in *Escherichia coli*. and *Staphylococcus Epidermidis*

Constant Variables and Control Group:
The negative control group had no antibiotics, it was a section of the petri dish with only *Escherichia coli* and *Staphylococcus Epidermidis* left in the dish. The positive control group was the 25% and 50% concentration of Amoxicillin. Constant Variables applied were: The type of bacteria used, the concentration of each antibiotic, time placed in the incubator, and the temperature of the incubator.

Background Information:
The care of acute burn wounds remains one of the most challenging healthcare problems worldwide. Approximately 500,000 people seek medical attention for burns every year in the United States, 40,000 of whom require hospitalization (CDC, 2017). Even with significant advancements in wound care over the last 50 years, burn wound infection is the leading cause of death in victims of extensive burn wounds. Multiple studies over the last decade have shown that 42%–65% of deaths in burn victims are attributable to bacterial infection. Also burn victims with infections have more than twice the mortality rate than that of uninfected patients. Unlike other types of injury, burn wounds induce metabolic and inflammatory alterations that predispose the patient to various complications such as serious wound infection. The most common bacteria causing burn wound infection are *Staphylococcus aureus* and *Escherichia coli* (WHO, 2014).

Antibiotics are used as a standard treatment against bacterial infections in burn wounds; however, because of the increasing problem of antibiotic resistance, new treatment options are being extensively researched, one of the most promising one of these is the use of flavonoids for treating burn wounds.

Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8000 individual compounds known so far. They possess a wide variety of antibiotic-like qualities such

as being antimicrobial, anti-oxidant, anti-cancer, anti-inflammatory and promoting wound healing. The basic structure of flavonoids is a diphenylpropane skeleton. That is, two benzene rings linked by a three-carbon chain that forms a closed pyran ring (heterocyclic ring containing oxygen, the C ring) with a benzenic A ring. The antibacterial activity of flavonoids seems to be related to their chemical structure, especially the number and positions of methoxy and hydroxyl groups.

Flavonoid subclasses, also known as flavonoids due to their chemical structure, show promise as potential antibiotic alternatives. One flavonoid that holds great promise as an antibiotic alternative is Quercetin. Quercetin, is a flavonoid found in fruits and vegetables. It has unique biological properties because of its unique structure. The structure of Quercetin is a pentahydroxyflavone having the five hydroxyl groups placed at the 3-, 3'-, 4'-, 5- and 7-positions. The five hydroxyl groups present in Quecertin's structure effectively increase its antibiotic activity because of their placement on Quecertin's aromatic rings. Quercetin and its derivatives showed significant antibacterial activity against some strains of bacteria, including *S. aureus*, methicillin-resistant *S. aureus* (MRSA), and *Staphylococcus Epidermidis* (Geoghegan & Wong, 2015).

Rutin is another example of a flavonol that holds great value as a potential antibiotic alternative. Rutin is structurally similar to Quercetin as it has the same chemical structure except with the addition of an added rutinose sugar. Rutin has demonstrated a profound degree of inhibition on growth of the bacteria *Escherichia coli* (Araruna et al., 2012). Hesperidin is an abundant and inexpensive by-product of citrus cultivation and is the major flavonoid in sweet orange and lemons. The structure of Hesperidin differs from Quercetin and Rutin because it is a flavonoid glycoside which has the chemical structure of a 3- hydroxyflavone backbone. Their diversity stems from the different positions of the phenolic - OH groups. They are distinct from flavanols (with "a ") such as quercetin another class of flavonoids. Naturally multifunctional Hesperidin has a great variety of biopharmaceutical activities, e.g. anticancer, anti-inflammatory, antioxidant and antimicrobial properties. Hesperidin has demonstrated antibacterial activity against *P. aeruginosa* (Celal Bayar University, 2016)

Traditional healing agents such as flavonoids assume a central role in wound care due to their clinical efficacy, simplicity, and affordability. These therapies represent a cost-effective alternative for the treatment of diverse difficult-healing wounds such as burn infections by providing a wide range of therapeutic effects that stimulate the healing process and improve the quality of the new skin. Traditional therapies can also be combined with modern clinical practices, biomaterials, and drugs, allowing the development of innovative therapeutic treatments that address important medical needs, such as minimize the bacterial resistance and reduce the healing time.

Description of Experiment

In this experiment various types of flavonoids (Quercetin, Rutin, Hesperidin) were tested on *Escherichia coli* and *Staphylococcus Epidermidis* to see if they were able to inhibit the growth of these bacteria. Amoxicillin was used as a positive control comparison to see if the flavonoids could perform to the same standard/efficacy as synthetic antibiotics. Another factor simultaneously researched in this experiment was using combinations of different flavonoids to see if synergistic interaction between flavonoids would increase the inhibition of growth on bacteria.

Hypothesis

Quercetin and any Quercetin combined compound when compared to Rutin and Hesperidin alone will be the most effective antibiotic alternative against *E. coli* and *S. epidermidis*, the two most common bacteria found in burn wound infections.The five hydroxyl groups present in Quercetin chemical structure enable it to possess enhanced antibacterial activity (Han, 2018). Therefore, Quercetin will have the highest success rate as an antibiotic alternative because of its strong antibacterial nature. Quercetin has also shown significant antibacterial activity against methicillin-resistant *S. aureus* (Geoghegan & Wang, 2015)

Materials:

This experiment requires the following materials:

- K-12 strain of *E. coli*
- Strain of *Staphylococcus Epidermidis*
- 50 Nutrient Agar Media Plates/Petri Dishes
- 875g of Amoxicillin (Pill form)
- 100g of Quercetin
- 100g of Rutin
- 100g of Hesperidin
- Antibiotic disks
- Tryptic Soy Broth
- Incubator
- Sterile Water
- Bunsen Burner
- Inoculating needle
- Inoculating loop
- Mortar Pestle
- Pipettes
- Ruler
- Ziploc Bags (Quart size)

Safety: Standard laboratory procedures must be followed. It is required to wear all necessary personal protective equipment, including long pants, closed-toed shoes, gloves and a lab coat. Additionally, all aseptic cleaning techniques must be followed — for example, the use of bleach or ethanol to clean all work surfaces. In the event of a bacterial spill, soak all areas of the suspected spill with ethanol/bleach and thoroughly wipe down when disposing of anything while in the lab, biohazard disposal bins must be used. Finally, be sure to label all plates and test tubes with name, date, bacteria type, and nutrient medium type.

Methods

This experiment will require 25 nutrient agar Petri dishes, prepared with *E. coli* bacteria and 25 nutrient agar Petri dishes prepared with *Staphylococcus Epidermidis*. The following procedure was used to create uncontaminated samples:

Isolating Streak Method and creating an uncontaminated sample of *E. coli* and *Staph Epi.* in TSB (tryptic soy broth)

i. First of all, prepare a petri dish of uncontaminated *E. coli* or *Staph Epi.* by using the following isolation streak technique/ quadrant streak method. Make sure to have four sterile inoculating loops, one for each quadrant.
ii. Begin by flaming the neck of the test tube containing the broth culture to create a sterile climate. Then dip the first inoculating loop into the broth culture and then begin streaking the plate by following the streaking pattern shown by 1 in diagram.
iii. Once done with the first streaking pattern, dispose of the inoculating loop in a biosafety bag. Continue following step ii for the three remaining loops and following the streak patterns shown in the diagram. Once finished, incubate the bacteria in an incubator set at 37 degrees Celsius for 24 hours.
iv. After the bacteria has cultured, place it under a fume hood to begin transfer from the petri dish to TSB (tryptic soy broth)
v. Using an inoculation needle, choose an isolated bacteria colony from the 4th quadrant. Then using the inoculation needle gently scrape the bacteria colony off, being cautious not to puncture the agar.
vi. Then taking the test tube with the TSB (tryptic soy broth) flame the neck of the test tube to create a sterile climate. Then take the inoculation needle with the isolated bacteria colony and gently scrape it on the side of the TSB test tube. Reflame the neck of the test tube and then cap the test tube
vii. Next, place the TSB test tube in an incubator shaker at 37 degrees Celsius for 24 hours, allowing the bacteria to culture for 24 hours. After 24 hours, the bacteria is now free from contamination and can be experimented on.

Data Analysis Methodology

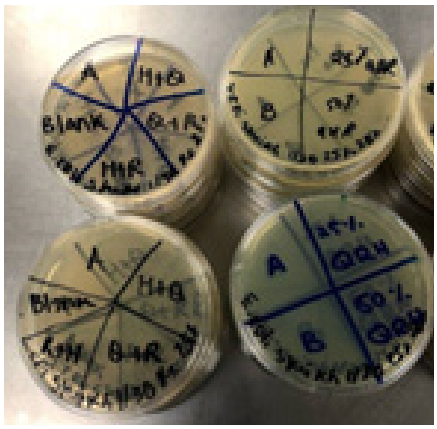
An Antibiotic Susceptibility Test will be used in order to accurately analyze the results from this experiment. The following rubric from Johnson and Case will be used:

Diameter of Zone of Inhibition (mm)	
10mm or less	Resistant
11mm-15mm	Intermediate
16mm or greater	Susceptible

1. **Susceptible (s):** A bacterial strain is said to be susceptible to a given antibiotic when it is inhibited in vitro by a concentration of this drug that is associated with a high likelihood of therapeutic success.
2. **Intermediate (i):** The sensitivity of a bacterial strain to a given antibiotic is said to be intermediate when it is inhibited in vitro by a concentration of this drug that is associated with an uncertain therapeutic effect.
3. **Resistant (r):** A bacterial strain is said to be resistant to a given antibiotic when it is inhibited in vitro by a concentration of this drug that is associated with a high likelihood of therapeutic failure. (Rodolf, 2008)

Experimental Procedure

1. Begin by first determining the weight based concentrations for each flavonoid type and Amoxicillin. Two concentrations will be made at 25% and 50%. The following weight based concentrations will be used for every 25g of flavonoid i.e Quercetin, Rutin, and Hesperidin 750 ml of water will be used to create the 25% concentration base. For Amoxicillin the same will be done for every 25g of Amoxicillin 750 ml of distilled water will be used. For the 50% weight based concentration 50g of each flavonoid type will be used and then diluted by 500g of distilled water, the same procedure is followed for Amoxicillin.
2. Once the weight-based concentrations are determined, the mixtures of flavonoids are created by using the concentrations available. To make a 25% concentration of Quercetin + Rutin, combine the 25% concentration of Rutin with the 25% Concentration of Quercetin. The same procedure can be followed for the remaining combinations of flavonoids.
3. Once the following concentrations are made: 25% Quercetin, 50% Quercetin, 25% Hesperidin, 50% Hesperidin, 25% Rutin, 50% Rutin, 25% Quercetin + Rutin, 50% Quercetin + Rutin, 25% Rutin + Hesperidin, 50% Rutin & Hesperidin, 25% Quercetin Hesperidin, 50% Quercetin Hesperidin, 25% Quercetin+Rutin+Hesperidin, and 50% Quercetin Rutin & Hesperidin, soak 5 antibiotic disks in each concentration for approximately 2 hours.
4. Once the 25% Amoxicillin and 50% Amoxicillin concentrations are created soak 60 antibiotic disks in each solution for approximately 2 hours
5. While the disks are soaking start labeling 50 petri dishes with name, date, type of bacteria, nutrient medium, trial number, and concentration of flavonoids and Amoxicillin.
6. While the concentrations are soaking, prepare the petri dishes by "flooding" the uncontaminated samples of *E. coli* or *Staph Epi.* Begin by taking a sterile cotton swab and streaking the petri dish in a zigzag motion all over the plate. Once flooding is accomplished the antibiotic disks are ready to be placed.
7. The following pattern was used for each petri dish.



8. Incubate overnight at 37 degrees Celsius and measure the zone of inhibition the next day.

Results

The effect of 25% concentration of Quercetin, Hesperidin, and Rutin in isolated and combined form on *E. coli*

	Zone of inhibition(mm)				
Flavonoid Type/ Control Group	Trial 1	Trial 2	Trial 3	Trial 4	Averages
25% Amoxicillin	22.2 mm	25.5 mm	21 mm	21.1 mm	22.45 mm
25% Quercetin	11.7 mm	12 mm	13.4 mm	12.2 mm	12.325 mm
25% Rutin	17.1 mm	14 mm	21 mm	20 mm	18.025 mm
25% Hesperidin	11 mm	12.4 mm	13.2 mm	12.5 mm	12.275 mm
25% Q+R	14.2 mm	17 mm	16 mm	18 mm	16.3 mm
25% R+H	15 mm	16.6 mm	17.2 mm	17.5 mm	16.575 mm
25% Q+H	11.7 mm	13.4 mm	11.4 mm	11.8 mm	12.075 mm
25% QRH	17 mm	20 mm	18.2 mm	19.1 mm	18.575 mm
Control	0 mm	0 mm	0 mm	0 mm	0 mm

Data Table 1 displays the results at 25% concentration of Quercetin, Hesperidin and Rutin in isolated and combined form on *E. coli*. The results show that Quercetin + Hesperidin + Rutin is the most effective compound out of all the flavonoid groups tested.

The effect of 25% concentration of Quercetin, Hesperidin, and Rutin in isolated and combined form on *S. epidermidis*

	Zone of inhibition(mm)				
Flavonoid Type/ Control Group	Trial 1	Trial 2	Trial 3	Trial 4	Averages
25% Amoxicillin	19.77 mm	26.1 mm	20.9 mm	22.9 mm	22.41 mm
25% Quercetin	14 mm	16.4 mm	12 mm	9.8 mm	13.05 mm
25% Rutin	16.1 mm	17.9 mm	21.1 mm	19.0 mm	18.525 mm
25% Hesperidin	13.8 mm	14.6 mm	14.2 mm	13.1 mm	13.925 mm
25% Q+R	20 mm	19 mm	21 mm	22.2 mm	20.55 mm
25% R+H	20.9 mm	16.77 mm	16.6 mm	16.1 mm	17.60 mm
25% Q+H	14.3 mm	12 mm	10 mm	11.1 mm	11.85 mm
25% QRH	23.1 mm	21.1 mm	21.3 mm	18.2 mm	20.9 mm
Control	0 mm	0 mm	0 mm	0 mm	0 mm

Data Table 2 displays the results at 25% concentration of Quercetin, Hesperidin and Rutin in isolated and combined form on *S. epidermidis*. The results show that Quercetin + Hesperidin + Rutin is the most effective compound out of all the flavonoid groups tested.

The effect of 50% concentration of Quercetin, Hesperidin, and Rutin in isolated and combined form on *E. coli*

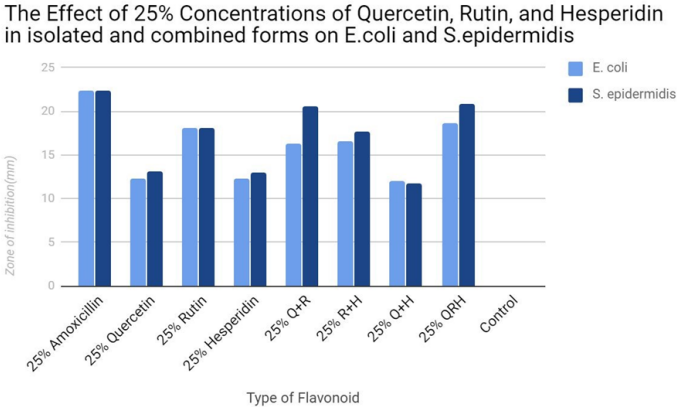
	Zone of inhibition(mm)				
Flavonoid/Control Group	Trial 1	Trial 2	Trial 3	Trial 4	Averages
50% Amoxicillin	30 mm	21.1 mm	25. 9 mm	28.99 mm	26.5 mm
50% Quercetin	10 mm	12 mm	11 mm	12.2 mm	11.3 mm
50% Rutin	21 mm	16.6 mm	14.1 mm	20 mm	17.9 mm
50% Hesperidin	11.1 mm	12 mm	14.3 mm	14.4 mm	12.875 mm
50% Q+R	18 mm	17.5 mm	14.2 mm	20 mm	17.425 mm
50% H+R	11 mm	11.4 mm	14 mm	13.7 mm	12.525 mm
50% Q+H	10 mm	11.3 mm	12.2 mm	14.4 mm	14.56 mm
50% QRH	22.2 mm	24.2 mm	22.1 mm	22.3mm	22.7 mm
Control	0 mm	0 mm	0 mm	0 mm	0 mm

Data Table 3 displays the results at 50% concentration of Quercetin, Hesperidin and Rutin in isolated and combined form on *E. coli*. The results show that Quercetin + Hesperidin + Rutin is the most effective compound out of all the flavonoid groups tested.

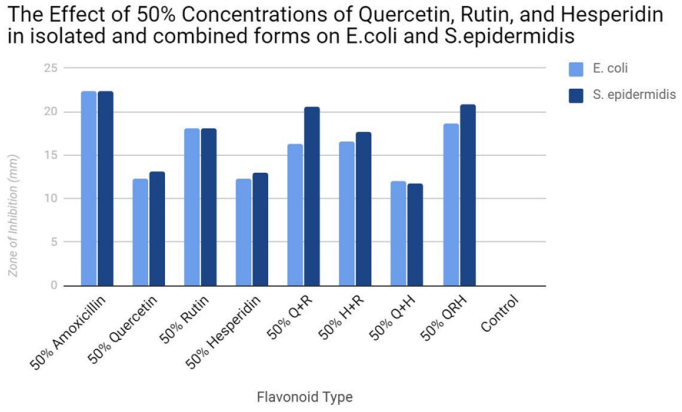
The effect of 50% concentration of Quercetin, Hesperidin, and Rutin in isolated and combined form on *S. epidermidis*

	Zone of inhibition (mm)				
Flavonoid/ Control Group	Trial 1	Trial 2	Trial 3	Trial 4	Averages
50% Amoxicillin	22.4 mm	26.8 mm	17.3 mm	21.6 mm	22.025 mm
50% Quercetin	11 mm	12.4 mm	10 mm	12 mm	11.35 mm
50% Rutin	14.8 mm	17.7 mm	15.2 mm	16.1 mm	15.95 mm
50% Hesperidin	11.8 mm	11.4 mm	11.46 mm	14.3 mm	12.24 mm
50% Q+R	15.6 mm	16.8 mm	17.13 mm	14.21 mm	15.935 mm
50% H+R	12.7 mm	11.8 mm	11.9 mm	11.8 mm	12.05 mm
50% Q+H	12.2 mm	11.8 mm	11.4 mm	11.9 mm	1182.5 mm
50% QRH	20.8 mm	17.6 mm	20.4 mm	16.45 mm	18.48 mm

Data Table 4 displays the results at 50% concentration of Quercetin, Hesperidin and Rutin in isolated and combined form on *S. epidermidis*. The results show that Quercetin + Hesperidin + Rutin is the most effective compound out of all the flavonoid groups tested.



Graph 1 displays the effect of 25% Quercetin, Rutin, and Hesperidin in isolated forms on both *E. coli* and *S. epidermidis*. As shown by the graph Amoxicillin was the most effective antibiotic while QRH was the most effective alternative antibiotic.



Graph 2 displays the effect of 25% Quercetin, Rutin, and Hesperidin in isolated and combined forms on both *E. coli* and *S. epidermidis*. As shown by the graph Amoxicillin is the most effective antibiotic overall, while QRH is the most effective antibiotic alternative.

Discussion of Results

To best analyze the data, the guidelines for an Antibiotic Susceptibility Test will be used. The following rubric from Johnson and Case will be used:

Diameter of Zone of Inhibition (mm)	
10mm or less	Resistant
11mm-15mm	Intermediate
16mm or greater	Susceptible

1. **Susceptible (s):** A bacterial strain is said to be susceptible to a given antibiotic when it is inhibited in vitro by a concentration of this drug that is associated with a high likelihood of therapeutic success.
2. **Intermediate (i):** The sensitivity of a bacterial strain to a given antibiotic is said to be intermediate when it is inhibited in vitro by a concentration of this drug that is associated with an uncertain therapeutic effect.
3. **Resistant (r):** A bacterial strain is said to be resistant to a given antibiotic when it is inhibited in vitro by a concentration of this drug that is associated with a high likelihood of therapeutic failure.

Out of every flavonoid group in isolated or combined forms, the Quercetin, Rutin, and Hesperidin combination worked best in both the 25% and 50% categories for both *E. coli* and *S. epidermidis*. The Quercetin, Rutin, and Hesperidin combination had the greatest zone of inhibition at an average of 22.4 mm for both concentrations, which classified it as part of the Susceptible category (ideal category with greatest success rate). Numerous other flavonoid groups placed into the Susceptible category, such as Rutin, the combination of Quercetin and Rutin, and the combination of Rutin and Hesperidin. Each of these groups listed had a zone of inhibition over 16mm. However, some flavonoid groups placed into the Intermediate category, such as Quercetin, Hesperidin, and the combination of Quercetin and Hesperidin. The following groups had an average zone of inhibition between 11mm-15mm. None of the flavonoids in either isolated or combined forms placed in the Resistant category, meaning that every flavonoid and flavonoid group had some type of antibacterial effect. Additionally, the results of the Antibiotic Susceptibility Test remained remarkably similar between the 25% and 50% concentration of the flavonoids regardless of the bacteria type.

Conclusion

Quercetin or any Quercetin combined compound when compared to Rutin and Hesperidin alone will be the most effective antibiotic alternative against *E. coli* and *S. epidermidis*, the two most common bacteria found in burn wound infections.The five hydroxyl groups present in the Quercetin chemical structure enable it to possess enhanced antibacterial activity (Han, 2018). Therefore, Quercetin will have the highest success rate as an antibiotic alternative because of its strong antibacterial nature. Quercetin has also shown significant antibacterial activity against methicillin-resistant *S. aureus* (Geoghegan & Wang, 2015). The hypothesis was proven correct as the combination of Quercetin, Rutin, and Hesperidin showed the most significant antibacterial activity for both *E. coli* and *S. epidermidis*, as compared to the other flavonoids. The QHR combination may have worked the best due to synergy occurring between the flavonoids. A study done by the University of Science and Technology of China tested synergistic action between Rutin, Morin, and Quercetin found that synergy occurs between the flavonoids results in a greater MIC (minimum inhibitory concentration). However, several errors may have occurred in this experiment, which could have resulted in decreased efficacy of the zone of inhibition. Unfortunately, contamination was determined in the 25% Amoxicillin group and the 25% Quercetin group, which resulted in Amoxicillin and Quercetin both being ineffective against *E. coli* and *S. epidermidis*. This contamination may have occurred due to the reason that the ziploc Bags which contained the various flavonoid concentrations may not have been 100% sterile, and the 25% Quercetin Concentration Bag leaked which may have compromised the concentration from being 100% sterile. Additionally, Quercetin, Rutin, and Hesperidin were extremely difficult to dilute with water as they did not dissolve properly in water, which may have contributed to the unexpected zone of inhibition results. Due to the solubility patterns of flavonols, Quercetin and Rutin would have easily dissolved in a substance such as ethanol. Flavonoid glycosides are readily soluble in methanol and ethanol (Pinho, 2012).

Additional Testing/ Future Research:

Combination of Flavonoids and Antibiotics

In this experiment, five flavonoids: Rutin, Hesperidin, Kaempherol, Quercetin, and Morin will be combined with synthetic antibiotics such as Amoxicillin, Cyproflaxin, and Tetracycline. A recent study done by the University of London found that combining Quercetin and Amoxicillin not only increased antibacterial activity six fold but also served as a possible solution for antibiotic resistance. In this experiment, combinations of flavonoids such as Rutin, Quercetin, and Amoxicillin will be tested to see the possible symbiotic interaction between a synthetic antibiotic and an alternative antibiotic and if that interaction results in a higher and more effective antibiotic.

Coronavirus disease and Flavonoids

Currently the world is panicking about an outbreak of respiratory illness caused by a new coronavirus first identified in Wuhan, Hubei Province, China. Coronaviruses (CoVs) have been rising targets of some flavonoids. The antiviral activity of some flavonoids against CoVs is presumed directly caused by inhibiting 3C-like protease (3CLpro). Coronaviruses (CoVs) are single-stranded RNA viruses with large, enveloped and positive senses that can infect both animals and humans (Kim et al, 2020). Further research could focus on the anti-viral properties of flavonoids against public health emergencies such as this Coronavirus pandemic.

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