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The Ratio of Linoleic to Oleic Acid in Sunflower Oil, and its Effects on Bacterial Growth

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ABSTRACT

This study aims to explore the extent to which varying the ratio of linoleic to oleic acid in processed sunflower affects the growth of *S. epidermidis* and *E. coli*. A modified Kirby-Bauer test was utilized to obtain quantitative results. Sunflower oil was diluted to various concentrations using Tween 80, then applied to bacteria inoculated in either pour-plate or spread-plate methods. All conditions tested were done in triplicate to conform to standards in antibacterial susceptibility testing.

Introduction

From use as an ingredient in food to cosmetics, sunflower seed oil, referred to as just sunflower oil, is widely utilized in many applications (Lin T-K et al. 2017). Its use as a skincare product in particular is rapidly being expanded on, where it is even recommended by the National Eczema Association as treatment for eczema (Hewett 2018). This popularity is partially attributed to the classification of sunflower oil as a type of moisturizer known as an emollient, which are highly sought-after as treatment for dermatological conditions such as atopic dermatitis, ichthyosis, and psoriasis (Moncrieff et al. 2013). Moisturizers in general have a wide variety of impacts on the skin, including maintaining skin barrier homeostasis, acting as antioxidants, and preventing inflammation (Lin T-K et al. 2017). Building on these effects, recent research has expanded to studying how moisturizers affect the growth of skin microorganisms.

The Skin Microbiome

The skin is colonized by a wide array of microorganisms, where skincare products are known to alter this ecology (Grice and Segre 2011). A study published in the International Journal of Cosmetic Science by Sfriso et al. (2019) further elaborates that imbalance of this microbiome can result in a variety of disorders, including pathological disorders such as acne and eczema, or non-pathological conditions such as sensitive and dry skin (Sfriso et al. 2019). As sunflower oil is commonly used in skincare, especially by those with preexisting skin conditions, its effect on skin microbiome is an aspect that should be accounted for when evaluating its use in the future.

Sunflower Oil and Microbial Growth

Not only does sunflower oil indirectly affect the skin microbiome as a moisturizer, it also has directly antibacterial properties attributed to its chemical makeup: sunflower oil consists of 90% linoleic and oleic acid, 10% palmitic and stearic acids, and trace amounts of other fatty acids (Awatif et. al 2014). Both linoleic and oleic acid are unsaturated fatty acids possessing antibacterial properties, yet the effect that the ratio of these acids have on the antibacterial efficiency of sunflower oil has yet to be determined (Neitzel, 2010; Zheng et al. 2005).

Bacterial Anatomy and Antibacterial Efficiency

The susceptibility of bacteria to certain antibiotics is dependent on the anatomy of the species tested. The most popular anatomical classification of bacteria is the Gram stain; gram-positive bacteria have a thicker cell wall, while gram-negative bacteria have thinner cell walls with an outer membrane and pores (Mai-Prochnow et al. 2016). This differing makeup results in distinct properties between the classifications, including antibiotic susceptibility (Mai-Prochnow et al. 2016). *S. epidermidis* is a gram-positive bacteria and *E. coli* is a gram-negative bacterium, where testing on both bacteria allows for generation of a better model of antibacterial effects (Lee and Anjum 2020).

S. epidermidis is a permanent, ubiquitous, generally symbiotic resident of human skin (Lee and Anjum 2020). In addition to acting as a representative of gram-positive bacteria in testing, its growth is also directly affected by application of skincare products due to its natural habitat. Not only does its presence have effects on the human host, where it may become an opportunistic pathogen, but its growth also strongly dictates the growth of other skin bacteria (Lee and Anjum 2020; Brown and Horswill 2020). Thus, due to the direct impacts topical products have on *S. epidermidis* and the implications of *S. epidermidis* growth, fluctuations in its population should be evaluated when using a skincare product such as sunflower oil that is known to have antibacterial effects.

Unlike gram-positive bacteria like *S. epidermidis*, gram-negative bacteria like *E. coli* make up a smaller portion of skin flora (Baron 1996). *E. coli* presence has more negative connotations due to its frequent isolation from skin infections (Petkovsek et al. 2009). Because of its differing Gram classification from *S. epidermidis*, its inclusion in the evaluation of antibacterial properties of sunflower oil allows for the effects of the oil on gram-negative bacteria to be obtained. It is essential that both Gram classifications are represented because the main components of sunflower oil, linoleic and oleic acid, are both unsaturated fatty acids that affect the ability of the bacteria to synthesize its cell wall (Neitzel, 2010; Zheng et al. 2005). As the cell walls of *S. epidermidis* and *E. coli* differ, it is important for both to be evaluated for a comprehensive view of the antibacterial effects of sunflower oil.

Thus, the inquiry "To what extent does the ratio of linoleic to oleic acid in sunflower oil affect the growth of *S. epidermidis* and *E. coli*?" may be raised. This study evaluates the relationship between the ratios of linoleic and oleic acid in sunflower oil and the oil's effects on *S. epidermidis* and *E. coli*. It is essential to take these effects into account when analyzing use of sunflower oil in skincare products due to its popularity as treatment for those with chronic skin conditions such as eczema (Hewett 2018). These populations with preexisting conditions are most vulnerable to bacterial infection, meaning that application of products known to directly affect bacterial growth such as sunflower oil should be closely monitored.

Review of Literature

Skin Microbiota and Skincare Product Usage

The skin microbiome has been studied for an extended period of time. Studies from decades ago have attributed imbalances in the microbiome to be the cause of many conditions, a 1977 study by Holland et al. that established the relation between acne and micrococcus bacteria being one such example (Holland et al. 1977). Even more recent studies regarding the skin microbiome are still primarily broad. One study conducted by Lee et al. (2017), scientists from Kunsan National University in Korea, tested the effects of skincare products on volunteers "belonging to high (HHG) and low (LHG) [skin] hydration groups," eventually concluding that "bacterial communities after cosmetic use were different from those in both LHG and HHG before the cosmetic use" (Lee et al. 2017). Although evaluation of solely facial bacteria narrowed the scope of the study, many aspects were broadly defined; "basic cosmetics" were given to participants, such as "skin softener (solubilized type), lotion (oil-in-water (O/W) emulsion type), essence (solubilized type), and cream (O/W emulsion type)," where specific ingredients in each of these were unspecified (Lee et al. 2017). Due to the wide diversity of skincare products available, the study only provided a generalized overview

of how skin bacteria react to application of cosmetics. A later study done in 2019 by Bouslimani et al. (2019) from the University of California, San Diego, also used a similar method of 16S rRNA sequencing to identify skin bacteria populations (Bouslimani et al. 2019; Lee et al. 2017). However, unlike Lee et al. (2017)'s study, Bouslimani et al. (2019) expands upon the areas of skin tested, sampling skin from the "face—upper cheek bone and lower jaw, armpit—upper and lower area, arm—front of elbow (antecubitis) and forearm," thus contributing to existing literature by quantifying bacterial concentrations on an increased area of skin. Bouslimani et al. (2019)'s investigation still falls into the same pitfall; products used were broadly defined as well, where compositions of the "deodorant for the armpits, soothing foot powder between the toes, sunscreen for the face, and moisturizer for front forearms" all had drastically varying active ingredients and compositions (Bouslimani et al. 2019). Both studies affirmed that skincare products affect the growth of skin bacteria, but this is a very broad conclusion (Bouslimani et al. 2019; Lee et al. 2017).

Plant Oil-specific Research

While plant oils such as sunflower oil are widely used in skincare, most scientific interest and clinical applications are directed to ozonised forms of these oils. Ozone is strongly bactericidal, with Ugazio et al. (2020) from the Department of Drug Science and Technology of the University of Torino attributing this property to ozone's ability to "induce the destruction of bacterial cell walls and the cytoplasmic membrane" (Ugazio et al. 2020). Treatment of oils with ozone creates "a reservoir of ozone that slowly releases into the skin," where this release results in "better bacteriostatic and bactericidal activity" of the oil (Ugazio et al. 2020). This quality of ozonated oils has thus resulted in its research overshadowing research of standard plant oils; antibacterial activity of ozonated sunflower oil has been evaluated since 2001, where Sechi et al. (2001)'s study published in the Journal of Applied Microbiology used agar dilution to determine the minimum concentration of ozonated sunflower oil needed to prevent visible growth of 6 genuses of bacteria. Attempts to quantify antibacterial activity of plain sunflower oil come later, with Aboki et al. (2012) from the National Research Institute for Chemical Technology, Nigeria, performing an investigation into sunflower oil's antibacterial activities using the Kirby-Bauer test (Aboki et al. 2012). Both of these studies failed to account for the exact composition of sunflower oil. As the concentration of oleic and linoleic acid in sunflower oil can vary drastically and the effects of these variations have not been examined, a gap in existing literature is thus presented (Huth et al. 2015; Dragan et al. 2008).

Application of topical antibiotics to combat harmful bacteria on the skin may become ineffective and costly with rising antibiotic resistance. As skincare products are applied regularly, exploration of how these products affect the skin microbiome is essential, especially for those who might have preexisting skin conditions. Because the effects of differing linoleic and oleic acid ratios on the antibacterial properties of sunflower oil have not been explored yet, the question is again raised: "To what extent does the ratio of linoleic to oleic acid in sunflower oil affect the growth of *S. epidermidis* and *E. coli*?"

Methods

An experimental method of inquiry was utilized for this study. This experimental method was chosen due to reliance on primary, quantitative data, with use of experimental controls. The technique chosen to collect quantitative data was also utilized in a similar study quantifying the antibacterial effects of standard sunflower oil (Aboki et al. 2012): the Kirby-Bauer test. This is the standardized method supported by the American Society for Microbiology for determination of bacterial susceptibility to certain compounds (Hudzicki 2009). The test involves inoculation of agar plates with a lawn of bacteria. Disks containing the compound tested are then placed on the agar, where inhibitory effects by the compounds are denoted by presence and size of a zone of inhibition around the disks where no bacterial growth is present. Generally, the larger the zone, the stronger the antibacterial effect of the compound tested (Hudzicki 2009).

The primary purpose of the Kirby-Bauer test is to present standardized, replicable data regarding antibacterial properties of various compounds.

Procedural Alterations

Sunflower oil classification can be specified into two widely used classifications: high linoleic and high oleic. High linoleic sunflower oil is defined as sunflower oil composed of approximately 80% linoleic acid, high oleic sunflower oil is defined as sunflower oil composed of approximately 70% oleic acid (Huth et al., 2015; Dragan et al., 2008). As the ratio of linoleic to oleic acid in these two types of sunflower oil vary drastically, use of both allows for insight on how the composition of sunflower oil affects bacterial growth. However, while the compositions of high linoleic and high oleic sunflower oils are defined and standardized, their exact constituents may vary marginally depending on the manufacturer (Huth et al. 2015; Dragan et al. 2008). As the purpose of my experiment was only to compare antibacterial action of high linoleic verses high oleic sunflower oil, not to define zones of inhibition based on the specific percent compositions, complete standardization was unnecessary for answering my primary research question. Replicability was maintained with precise measurements, and alterations from the standard were done to suit a public school laboratory and for a more comprehensive view of the oils being tested. The alterations are as follows: use of nutrient agar in place of Mueller-Hinton agar, differed inoculation standard, and lengthened duration of the trial period from 24 hours to 72 hours of growth.

As *S. epidermidis* and *E. coli* both grow readily and are nonfastidious, any agar will promote their growth. Due to its availability in my school laboratory, nutrient agar was used in place of Mueller-Hinton agar. The cell density that a plate is inoculated with also matters when measuring bacterial growth, where a 0.5 McFarland standard is the general concentration used in Kirby-Bauer testing (Hudzicki 2009). Preparation of the standard either requires visual judgement of a concentration or use of a spectrophotometer for measurement (Hudzicki 2009). The former is prone to human error, and the latter was incompatible with the bacteria tested; *S. epidermidis* is a coccus bacteria that lacks flagella, meaning that it sinks in solution and does not remain suspended long enough for a proper spectrophotometric reading (Lee and Anjum 2020). An alternative inoculation standard used will be described later.

Linoleic and oleic acid are unsaturated fatty acids that inhibit synthesis of fatty acids that are used for growth of bacterial membranes (Neitzel 2010; Zheng et al. 2005). As sunflower oil's mechanism of action involves disturbance of growth by affecting synthesis of key products rather than immediate, fatal disturbance of cellular membranes, it was hypothesized that long-term evaluation over a duration of 72 hours would produce a more realistic model of growth. Additionally, sunflower oil is used long-term as a moisturizer rather than as a one-time treatment, further adding to the validity of this choice.

Materials Used

Supplies such as forceps, micropipettes, eppendorf tubes, an autoclave, stir plates, distilled water, erlenmeyer flasks, graduated cylinders, paraffin, inoculating loops, bunsen burners, 85mm petri dishes, and beakers were already available for use in this study. Purchases of materials outside of what is readily available in a general laboratory that might be necessary for replication are denoted below (Table 1).

Table 1. Materials Used

Product Name	Company	Quantity	Cost
Sunflower Oil - Refined - Mid Oleic (High Linoleic)	Blossom Bulk	1 container	$15.92
Sunflower Oil - Refined - High Oleic	Blossom Bulk	1 container	$19.20
POLYSORBATE 80 T-MAZ 80 TWEEN 80 SOLUBILIZER SURFACTANT & EMULSIFIER PURE 4 OZ	H&B Oils Center	1 container	$7.29
Staphylococcus epidermidis, Living, Nutrient Broth	Carolina	1 tube	$12.25
Escherichia coli, K-12 Strain, Living, Nutrient Broth, Tube	Carolina	1 tube	$12.25
Nutrient Broth, Prepared Media Tubes, Pack of 10	Carolina	1 pack	$22.20
Nutrient Agar, Dehydrated Medium, 100 g	Carolina	1 pack	$43.45
United Scientific FPR150 FILTER PAPER, CIRCULAR, GRADE 1, 15 CM DIAMETER 100/PK	United Scientific	1 pack	$7.68

Preparation of Oils

While certain concentrations of sunflower oil may have strong antibacterial effects, their ease of diffusion into agar will also affect their eventual zone of inhibition (Hudzicki 2009). Dilution of the oils allowed for evaluation of the best concentration for antibacterial effects. This is important as these oils are often diluted or combined with other ingredients in skincare products. Thus, differing concentrations of high linoleic and high oleic sunflower oils were prepared. A detergent was used to emulsify oil with distilled water. Tween 80 is a detergent commonly used in cosmetics and considered to have very minimal, if any, antibacterial effects at low concentrations, making its use ideal for this experiment (Nielsen 2016). This choice was made based on the methods of a study conducted by faculty from the Department of Pharmaceutical Technology the University of Sumatera Utara, Indonesia, which utilized both Tween 80 as a detergent and sorbitol as a stabilizer to create a stable nanoemulsion of sunflower oil into water (Arianto and Cindy 2019). As the purpose of my study only required creation of temporary dilutions, Tween 80 alone was utilized for its properties as a detergent. A 0.5% volume-to-volume (v/v) ratio of Tween 80 to sunflower oil was determined to be the lowest ratio achievable for emulsion.

Table 2. Percent composition of oil/water emulsions

Compounds	25%	50%	75%	100%	Tween 80 Control
Tween-80 (mL)	0.125	0.25	0.375	0	0.375
Oil (mL)	25	50	75	100	0
Water (mL)	75	50	25	0	100

Four concentrations of solutions were created: 25%, 50%, 75%, and 100%, where the percentage specifies the v/v ratio of sunflower oil to water in each mixture (Table 2). Tween 80 was pipetted into a sterilized erlenmeyer flask with sunflower oil, vortexed, and transferred to a stir plate, where distilled water was added slowly until the required ratio was achieved and a visually homogenous sample was obtained (Fig. 1). Samples were labeled, covered in parafilm to prevent contamination, and stored at room temperature.

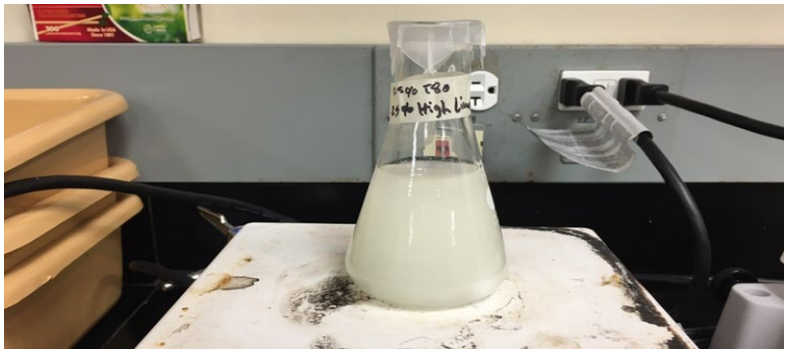


Figure 1. A complete sunflower oil/water emulsion

Pre-Experimental Preparation

Prior to execution of the main procedure, agar, diffusion disks, and broth needed to be obtained and sterilized. Nutrient agar was formulated according to instructions given on the container by Carolina. Broth was purchased from Carolina as well. 6mm disks were created by hole-punching filter paper. The agar, broth, and disks were autoclaved for sterilization. Additionally, preparation of the bacteria had to be done in advance; *E. coli* and *S. epidermidis* were removed from slant cultures, quadrant-streaked on a plate, and incubated for 37 degrees Celsius for next-day use to ensure all bacteria were fresh.

Main Procedure

Suspension of *S. epidermidis* and *E. coli* in broth had to be created for inoculation. This involved use of an inoculating loop to trace a 1 mm trail on the surface of the 24-hour plates to collect colonies. This measurement ensures that roughly the same cell density of bacteria was placed on each plate and serves as a substitute for the 0.5 McFarland standard. The loop was then swirled around 1 mL of nutrient broth in an Eppendorf tube until the bacteria were dislodged. 24 of these tubes were created, one for each petri dish to be created. Tubes would be vortexed prior to inoculation to ensure an even inoculation solution. Antiseptic procedures were followed to avoid contamination, involving sterilization of the loops with Bunsen burners between each use.

Afterwards, 24 85mm diameter plates of 4mm deep nutrient agar were poured. This allows for triplicates to be performed for each of the study parameters, similar to setups of past studies (Aboki et al. 2012); triplicates are the standard for antibacterial testing. Plates were labeled according to the sunflower oil being tested— high oleic or high linoleic— and the bacteria being tested— *E. coli* or *S. epidermidis*. Plates were inoculated in two ways: spread-plate and pour-plate. The spread-plate method (SPM) involved spread of the 1mL broth inoculum across the solidified nutrient agar to establish even surface growth. The pour-plate method (PPM) involved addition of 1mL of broth inoculum at the same time as liquid agar, where then the dish is swirled to establish growth both on the surface and within the medium (Sanders 2012). This method better mimics bacterial growth within the skin; the skin microbiome is present and uniform in both the deep dermis and superficial epidermis (Bay et al. 2020). Both methods were utilized to gain a more comprehensive evaluation of the effects of the sunflower oil on both surface growth and deeper growth of bacteria.

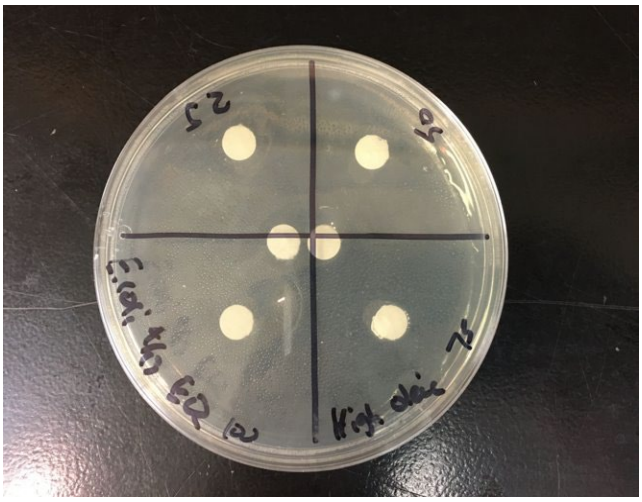


Figure 2. Standard plate setup pre-inoculation

Sunflower oil disks were created the day of the experiment; pre-prepared solutions of Tween 80, distilled water, and sunflower oil in the four concentrations denoted prior (Table 2) were placed on a stir plate again to ensure complete emulsion, where filter paper disks were dipped in to ensure uniform spread of the solution and allowed to dry on glass slides sterilized by ethanol. Plates were then divided into quadrants (Fig. 2); one of each disk would be laid down per quadrant, with a dry control and Tween 80 control in the middle for a total of 6 disks per plate. The disks were placed with autoclaved forceps, with the same forceps being used for each species. Finally, plates were taped to prevent contamination and placed in the incubator at 37 degrees Celsius. Composition of the plates created for this experiment are denoted below (Table 3).

Table 3. Plate setups

Sunflower Oil Type	Bacterial Species	Method	Number of Plates
High Oleic	*E. coli*	Pour-plate	3
High Oleic	*E. coli*	Spread-plate	3
High Oleic	*S. epidermidis*	Pour-plate	3
High Oleic	*S. epidermidis*	Spread-plate	3
High Linoleic	*E. coli*	Pour-plate	3
High Linoleic	*E. coli*	Spread-plate	3
High Linoleic	*S. epidermidis*	Pour-plate	3
High Linoleic	*S. epidermidis*	Spread-plate	3
Total Plates:			24

Results

All conditions were done in triplicate (Table 3). Plates were removed from the incubator every 24 hours and the distance across the inhibition zone, including the paper disk, was measured. This is the diameter of the zone of inhibition. If no zone was present, the zone of inhibition was recorded as 0. The following tables and figures denote the zones of inhibition measured for the types of sunflower oil tested after incubation.

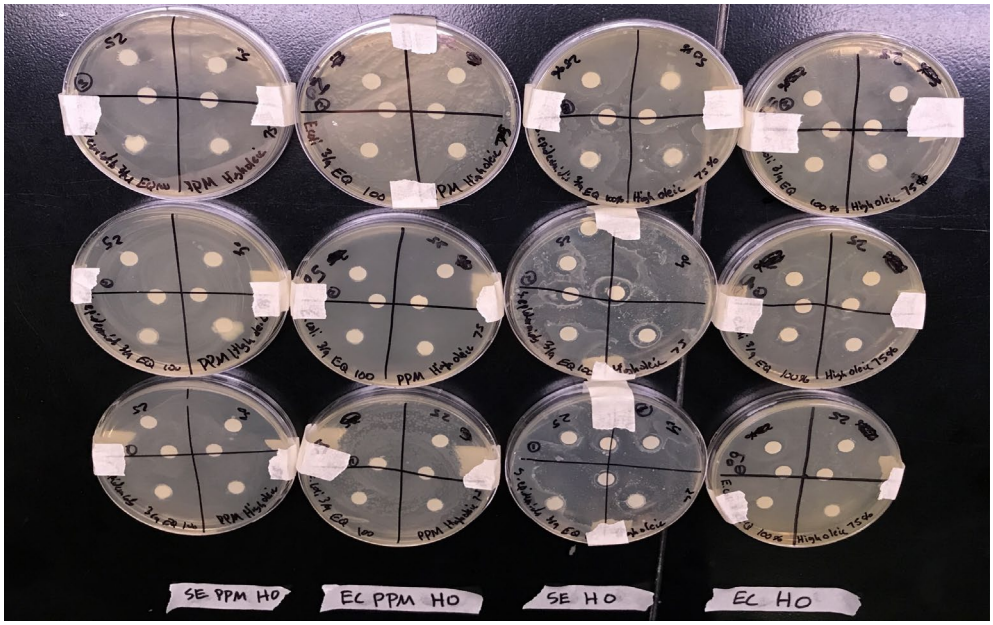


Figure 3. High oleic sunflower oil plates after 24 hours of growth

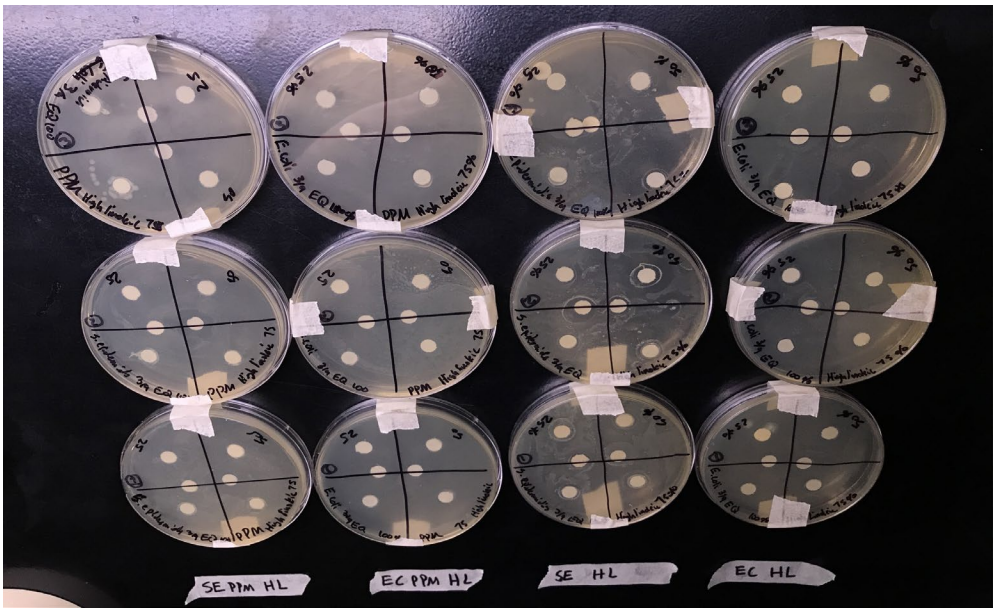


Figure 4. High linoleic sunflower oil plates after 24 hours of growth

For ease of organization, columns in the following figures will be in the same format shown prior (Fig. 3-4); plates in the same column are of the same conditions, which are abbreviated at the bottom label in the order of bacterial species, method of inoculation, and oil tested. For instance, the first column, SE PPM HO, is of *S. epidermidis* inoculated using the pour-plate method with differing concentrations of high oleic sunflower oil disks. Tables use abbreviations as well; for example, EC 1 (SPM) denotes the first plate of E. coli inoculated using the spread-plate method. Spots from bacterial contamination or growths from fungal contamination were evident on some plates (Fig. 4). Only plates where contamination affected the zones of inhibitions measured were recreated for a repeat trial, represented by the gray cells in the tables below.

Table 4. Zones of inhibition for high oleic sunflower oil, 24 hours

Plate	Dry	Tween	25%	50%	75%	100%
EC 1 (SPM)	0	0	0	0	0	0
EC 2 (SPM)	0	0	0	0	0	0
EC 3 (SPM)	0	0	7	7	0	0
SE 1 (SPM)	0	8	8	8	8	8
SE 2 (SPM)	0	10	0	0	0	0
SE 3 (SPM)	0	10	8	8	12	0
EC 1 (PPM)	0	0	0	0	0	0
EC 2 (PPM)	0	0	0	0	0	0
EC 3 (PPM)	0	0	0	0	0	0
SE 1 (PPM)	0	0	0	0	0	0
SE 2 (PPM)	0	0	0	0	0	0
SE 3 (PPM)	0	0	0	0	0	0

Table 5. Zones of inhibition for high linoleic sunflower oil, 24 hours

Plate	Dry	Tween	25%	50%	75%	100%
EC 1 (SPM)	0	0	8	0	7	0
EC 2 (SPM)	0	0	0	0	0	0
EC 3 (SPM)	0	0	0	0	0	0
SE 1 (SPM)	7	8	12	10	10	10
SE 2 (SPM)	8	12	8	12	10	8
SE 3 (SPM)	0	0	14	12	8	8
EC 1 (PPM)	0	0	0	0	0	0
EC 2 (PPM)	0	0	0	0	0	0
EC 3 (PPM)	0	0	0	0	0	0
SE 1 (PPM)	0	0	0	0	0	0
SE 2 (PPM)	0	0	0	0	0	0
SE 3 (PPM)	0	0	0	0	0	0

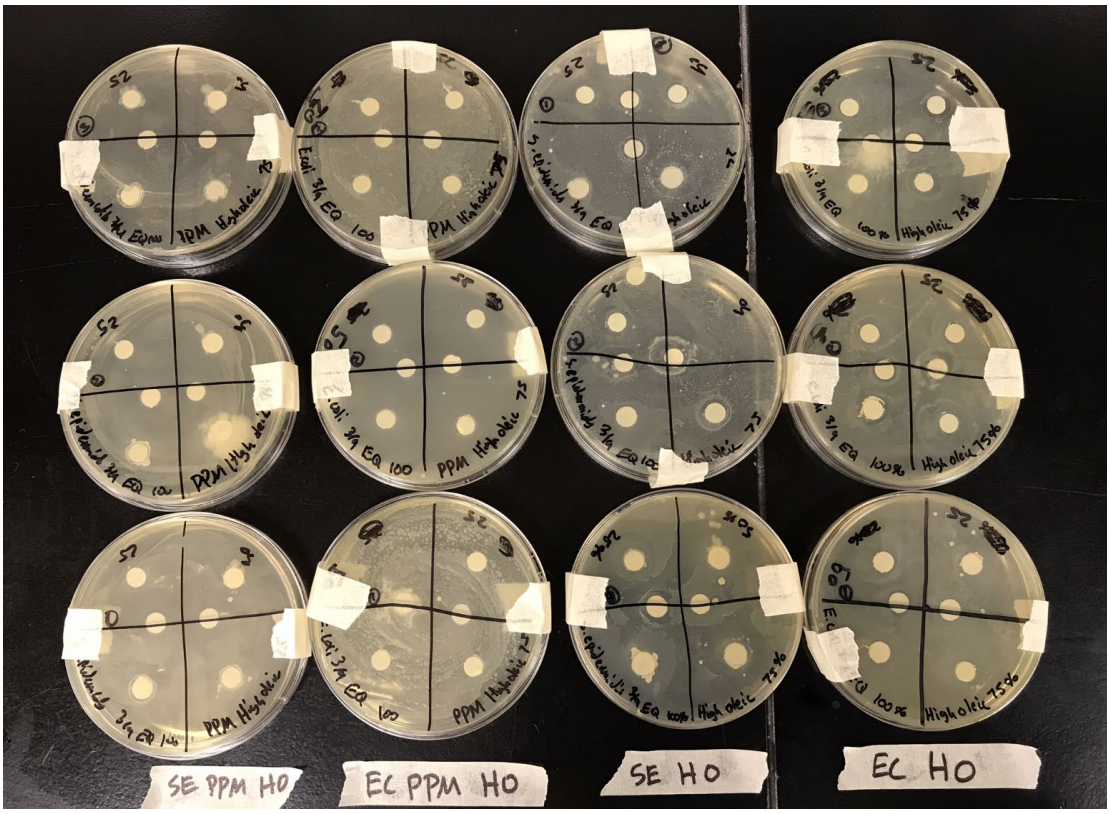


Figure 5. High oleic sunflower oil plates after 48 hours of growth

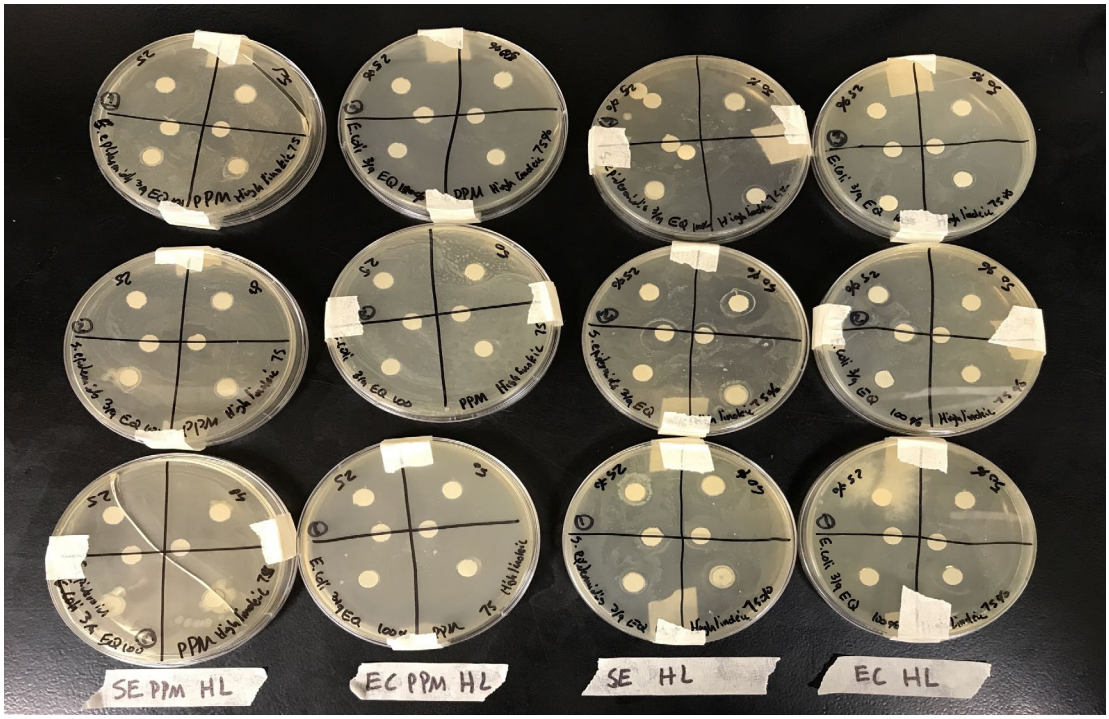


Figure 6. High linoleic sunflower oil plates after 48 hours of growth

Table 6. Zones of inhibition for high oleic sunflower oil, 48 hours

Plate	Dry	Tween	25%	50%	75%	100%
EC 1 (SPM)	8	8	16	12	22	12
EC 2 (SPM)	8	8	14	14	20	12
EC 3 (SPM)	0	0	15	11	18	15
SE 1 (SPM)	0	10	12	10	14	8
SE 2 (SPM)	10	12	14	8	18	8
SE 3 (SPM)	0	10	13	14	12	11
EC 1 (PPM)	0	0	0	0	0	0
EC 2 (PPM)	0	0	0	0	0	0
EC 3 (PPM)	0	0	0	0	0	0
SE 1 (PPM)	0	0	0	0	0	0
SE 2 (PPM)	0	0	0	0	0	0
SE 3 (PPM)	0	0	0	0	0	0

Table 7. Zones of inhibition for high linoleic sunflower oil, 48 hours

Plate	Dry	Tween	25%	50%	75%	100%
EC 1	0	0	10	10	10	12
EC 2	0	10	12	14	16	12
EC 3	0	0	10	18	16	10
SE 1	8	8	14	22	26	10
SE 2	8	10	14	20	28	10
SE 3	0	0	16	23	22	8
EC 1 (PPM)	0	0	0	0	0	0
EC 2 (PPM)	0	0	0	0	0	0
EC 3 (PPM)	0	0	0	0	0	0
SE 1 (PPM)	0	0	0	0	0	0
SE 2 (PPM)	0	0	0	0	0	0
SE 3 (PPM)	0	0	0	0	0	0

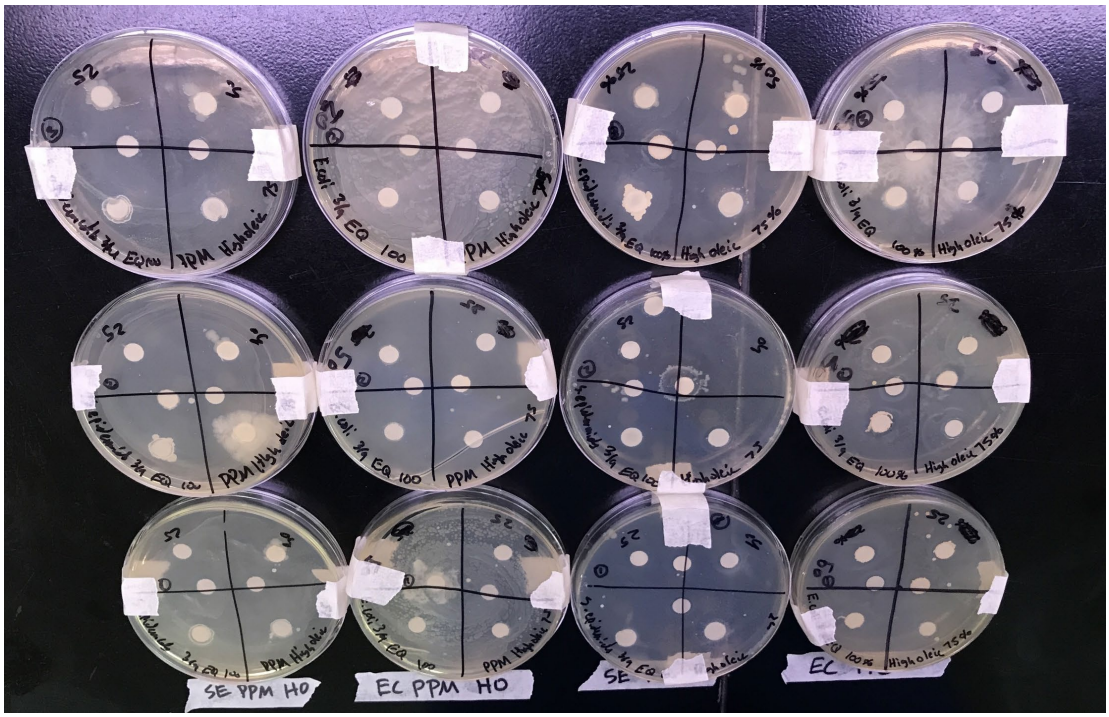


Figure 7. High oleic sunflower oil plates after 72 hours of growth

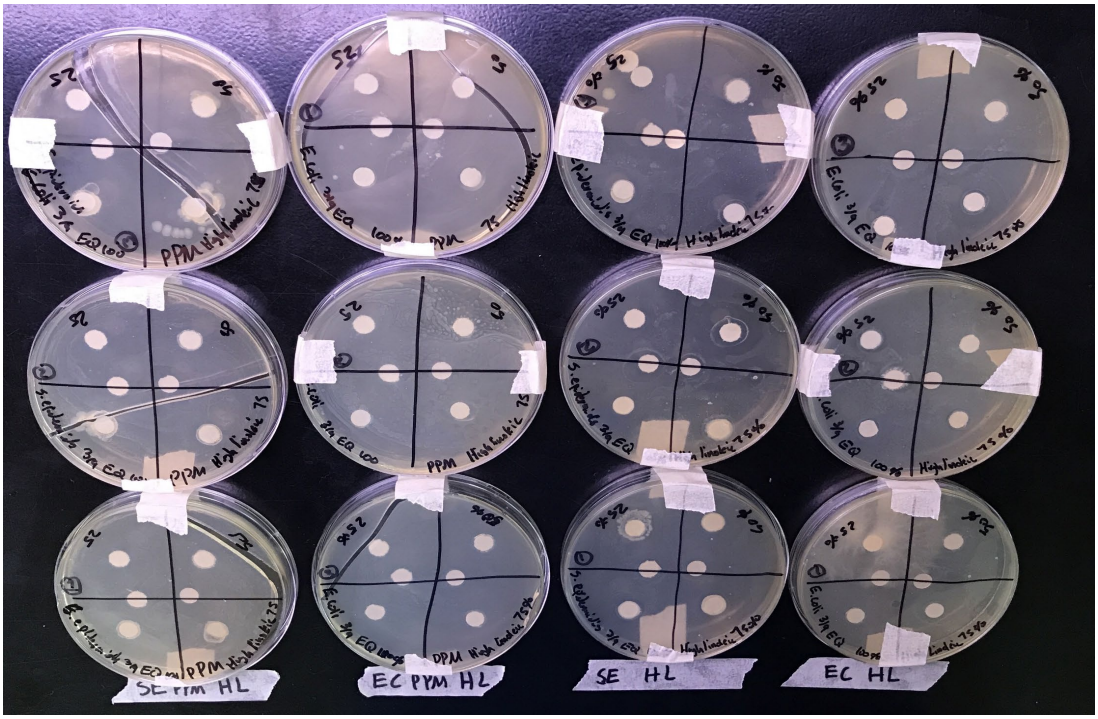


Figure 8. High linoleic sunflower oil plates after 72 hours of growth

Cracks in the agar present at 48 hours and 72 hours (Fig. 5-8) were due to dehydration caused by growth of bacteria. I took note of this, where the zone of inhibition was measured so that the crack was not included.

Table 8. Zones of inhibition for high oleic sunflower oil, 72 hours

Plate	Dry	Tween	25%	50%	75%	100%
EC 1 (SPM)	8	8	16	12	32	12
EC 2 (SPM)	10	8	16	14	24	14
EC 3 (SPM)	0	0	15	11	22	16
SE 1 (SPM)	0	10	18	10	16	8
SE 2 (SPM)	12	12	14	8	18	8
SE 3 (SPM)	0	10	16	14	13	12
EC 1 (PPM)	0	0	0	0	0	0
EC 2 (PPM)	0	0	0	0	0	0
EC 3 (PPM)	0	0	0	0	0	0
SE 1 (PPM)	0	0	0	0	0	0
SE 2 (PPM)	0	0	0	0	0	0
SE 3 (PPM)	0	0	0	0	0	0

Table 9. Zones of inhibition for high linoleic sunflower oil, 72 hours

Plate	Dry	Tween	25%	50%	75%	100%
EC 1 (SPM)	0	0	15	16	11	12
EC 2 (SPM)	0	10	12	14	16	12
EC 3 (SPM)	0	0	10	18	16	10
SE 1 (SPM)	8	8	14	22	26	10
SE 2 (SPM)	10	12	14	20	28	10
SE 3 (SPM)	0	0	16	23	24	8
EC 1 (PPM)	0	0	0	0	0	0
EC 2 (PPM)	0	0	0	0	0	0
EC 3 (PPM)	0	0	0	0	0	0
SE 1 (PPM)	0	0	0	0	11	0
SE 2 (PPM)	0	0	0	0	10	0
SE 3 (PPM)	0	0	0	0	5	0

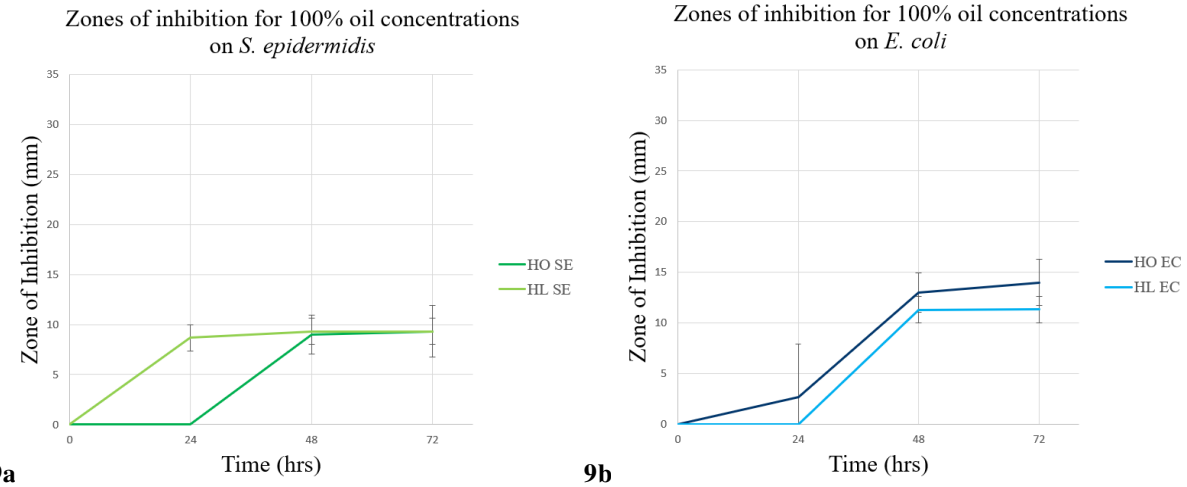
Discussion

Results from the Kirby-Bauer test do not yield absolute recommendations of use of high linoleic over high oleic sunflower or vice versa; results are based on the zone of inhibition, in which there is no bacterial growth, which does not equate to positive effects on skin. Antibacterial effects may result in an imbalance of the skin microbiome, leading to skin disorders. Evaluations from this study mean to provide insight into mechanisms of actions of the oil and how ratios of linoleic to oleic oil affect antibacterial activity at different dilution concentrations, creating a solid foundation

for future research or in vivo trials, and answering the research question of "To what extent does the ratio of linoleic to oleic acid in sunflower oil affect the growth of *S. epidermidis* and *E. coli*?"

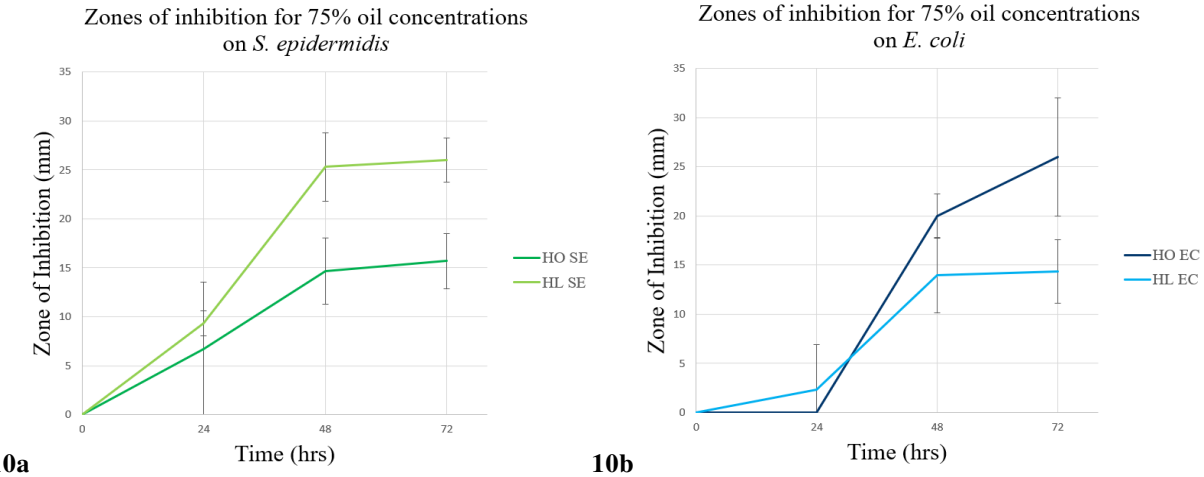
The trials were done in triplicate to allow for creation of reliable standard deviations (SD). The standard error of the mean (SEM), how accurately the sample mean represents the true population mean, was then calculated from the standard deviation (Altman 2005). SEM is calculated as the SD divided by the square root of the sample size. The SEM is then multiplied by 1.96 to achieve a 95% confidence interval (CI), meaning that there is a 95% chance that the CI given will be where the true mean of the population lies (Altman 2005). The 1.96 is rounded to 2 for neatness of expression, where error bars are represented by ±2SEM in the following figures. CI bars are not foolproof, but for the larger quantity of data collected in this experiment, overlaps in these bars can be judged as an indication of statistical insignificance. Further statistical analysis would be worthwhile for experiments with large sample sizes for single conditions, but as the purpose of this experiment was to find general relationships and propose parameters for future direction, CI calculations are adequate.

The following graphs are abbreviated where HO stands for high oleic, HL stands for high linoleic, SE stands for *S. epidermidis,* EC stands for *E. coli*. All graphs report data from the spread-plate method, as almost no antibacterial activity was present on the pour-plate setups.



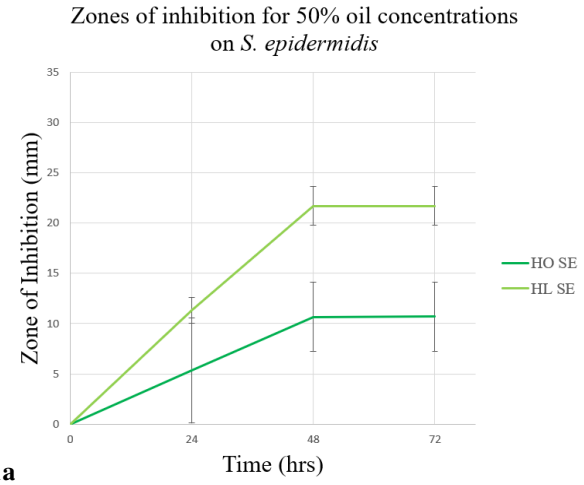
9a **9b**

Figure 9. Measured zones of inhibition over 72 hours for 100% sunflower oil concentration concentrations on a) *S. epidermidis* growth and b) *E. coli* growth

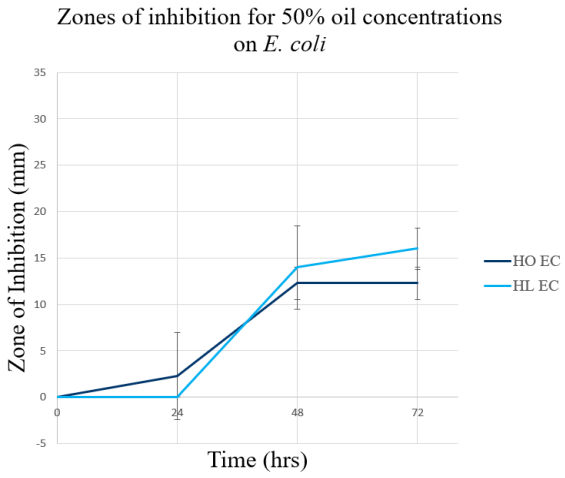


10a **10b**

Figure 10. Measured zones of inhibition over 72 hours for 75% sunflower oil concentration concentrations on a) *S. epidermidis* growth and b) *E. coli* growth

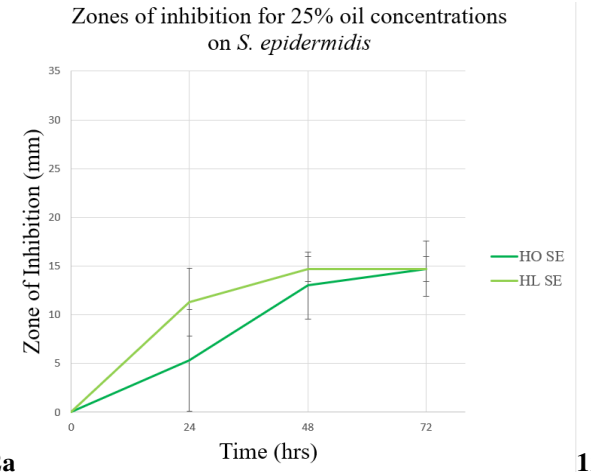


11a

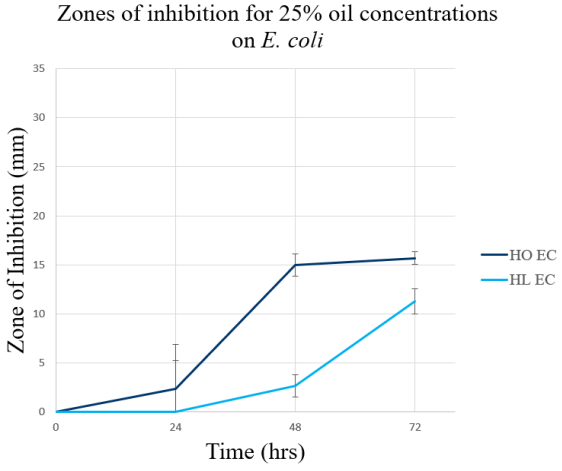


11b

Figure 11. Measured zones of inhibition over 72 hours for 50% sunflower oil concentration concentrations on a) *S. epidermidis* growth and b) *E. coli* growth



12a



12b

Figure 12. Measured zones of inhibition over 72 hours for 25% sunflower oil concentration concentrations on a) *S. epidermidis* growth and b) *E. coli* growth

Overall analysis

Antibacterial effects for all oils become more apparent with time, as indicated by increasing zones of inhibitions for all figures from 0 to 72 hours. Error bars also tend to overlap at 24 hours (Fig. 12). This is due to the method of measurement; an oil may inhibit growth of bacteria underneath the disk but diffuse poorly to surrounding agar. If no zone of inhibition is present around the disk, the zone of inhibition is recorded as zero, even if antibacterial effects are exerted on the growth below the disk (Hudzicki 2009). Such variance contributes to the higher errors found at 24 hours.

The antibacterial strength of the sunflower oils relative to each other mostly remained stable over time as well, where one oil would generally demonstrate consistently higher antibacterial activity on one species of bacteria than the other. There are exceptions present (Fig. 11b); prior measurements (Fig. 9-10) demonstrate that high oleic oil is more effective on E. coli at 48-72 hours, but this relation flips later on (Fig. 11b). This indicates the existence of optimal dilutions for the oils, where over- or under- dilution decreases antibacterial activity. This would mean that

50% dilution would be the optimal dilution for high linoleic sunflower oil for it to have the strongest effects on *E. coli* when compared to high oleic oil (Fig. 11b). Thus, the extent to which the ratio of linoleic to oleic acid in sunflower affects growth of *S. epidermidis* and *E. coli* varies with how diluted the oils are as well, where the bacteria most affected can switch upon dilution.

Analysis by dilution

At 100% concentration (Fig. 9) there is no significant relationship between the ratio of linoleic to oleic acid in the sunflower oil and antibacterial activity on *E. coli*. The same largely applies to *S. epidermidis*, where the only significant difference is at 24 hours, with high linoleic presenting itself as significantly faster acting than high oleic oil on S. epidermidis.

Dilution of the sunflower oils to a 75% oil to water ratio results in more pronounced antibacterial effects from that of 100% concentration; at 75% dilution, high oleic oil demonstrates significantly more effective activity on *E. coli* than does high linoleic oil at 48 and 72 hours, indicated by non-overlapping error bars (Fig. 10b). The same separation at 48-72 hours for the oils on *S. epidermidis* can be seen, except high linoleic oil has greater activity than high oleic oil (Fig. 10a). This relationship between a bacteria's gram stain and composition of sunflower oil has not been previously documented.

Further dilution of sunflower oil to 50% concentration shows a similar pattern to what was recorded from 100% to 75%; antibacterial effects are even more clearly pronounced, where high linoleic oil demonstrates statistically higher activity than than high oleic oil on *S. epidermidis* across the whole range of time (Fig. 11a). As stated before, this is where deviation occurs from the general trend of high oleic oil demonstrating greater antibacterial activity on *E. coli* than high linoleic oil. Dilution to 25% (Fig. 12b) restores the previous trend (Fig. 9-10) of high oleic acid having greater antibacterial activity on on *E. coli*, yet the previous trend of high linoleic oil demonstrating greater antibacterial activity on S. epidermidis than high oleic acid (Fig. 10-11) is disturbed, with antibacterial comparisons being statistically insignificant for all times.

Implications of trends

The figures indicate that high linoleic acid is generally more effective in inhibiting growth of the gram-positive *S. epidermidis*, especially at 50% concentration. High oleic acid is generally more effective at inhibiting growth of the gram-negative *E. coli*, but displays less predictable antibacterial effects across dilutions. The ratio of linoleic to oleic acid in sunflower oil also affects its diffusion and thereby effectiveness of the oil; more pronounced antibacterial effects of linoleic acid after dilution to 75% and 50% could imply that a higher composition of linoleic acid results in greater difficulty in oil diffusion across the agar in its pure state, with the decrease in effectiveness compared to oleic acid after dilution to 25% indicating that excessive dilution of oil reaches a point where addition of water no longer helps the oil diffuse, but decreases its effectiveness as there is a lesser concentration of oil overall in the disk.

All the analysis thus far has been on plates created with the spread-plate method; at 72 hours, only the *S. epidermidis* pour-plates had measurable antibacterial effects from 75% high linoleic oil (Table 9). Spread-plates had bacterial growth concentrated on the surface of the agar, while pour-plates had bacterial growth across all agar depths. This again illustrates the impact of diffusion on the antibacterial effects of the oils, where high linoleic oil at 75% was likely the best combination for antibacterial effects and adequate diffusion for such effects to be felt. The timing of these results also show that antibacterial effects on the skin may be superficial in the short run; for long-run changes in skin microbiome, the oil will have to diffuse to the deeper dermis to have an effect across all layers of skin. Thus, from the antibacterial effects observed, my initial research question of "To what extent does the ratio of linoleic to oleic acid in sunflower oil affect the growth of *S. epidermidis* and *E. coli*?" can be answered; varying the ratio of linoleic to oleic acid in sunflower oil does result in statistically significant different effects on the growth of *S. epidermidis* and E. *coli*, where the extent of this significance is dependent on the dilution of the oil, the gram stain of the bacteria tested, and how easily it diffuses across a medium.

Limitations

Although the experiment was conducted in sterile environments, additional measures may be taken in future recreation or alterations to the procedure. Error may result from contamination of the plates; this contamination was not significantly detrimental to the experiment, but its elimination would provide clearer results. This includes filtration of solutions used to remove contaminants and autoclaving additional equipment.

SEM becomes more accurate as sample size grows, meaning that additional trials would clarify data. For instance, at 25% oil concentration (Fig. 12a) the trend of high linoleic oil being comparatively more effective on *S. epidermidis* is displayed by the mean, yet CI bars still overlap, indicating statistical insignificance. It would be beneficial to have these areas clarified in future research. Tween 80 controls also demonstrated antibacterial effects (Tables 4-9). This was unavoidable; other detergents would have had stronger antibacterial effects than the Tween. All measurements for Tween 80 controls were lower than those of the sunflower oil disks.

The Kirby-Bauer test represents a way to measure antibacterial susceptibility in vitro; in vivo experimentation would be strongly beneficial if these oils are to be evaluated in the future for use by those with skin disorders. This allows for additional variables to be factored into the data, as real conditions are not as controlled as what is portrayed in a sterile lab.

Conclusion

Numerous routes can be taken for further experimentation. Through the skincare lens, there could be benefits to researching circumstantial use of one oil over another. For instance, conditions like eczema exacerbated by gram-positive bacteria might benefit more if treated with high linoleic oil, which demonstrates stronger antibacterial effects than high oleic oil on the gram-positive *S. epidermidis*. The converse may be said for treatment of skin disorders that are caused by gram-negative bacteria. Existing research involving sunflower oil such as Sechi et al. (2001)'s study on ozonated sunflower oil effects on bacterial growth can also be recreated with greater emphasis on the composition of the sunflower oil used. While such studies have not paid close attention to the composition of sunflower oil, results from my study show that the composition of sunflower oil does significantly affect growth of bacteria and should therefore be kept in mind in future studies.

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