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*Original Article / Araştırma Makalesi*

# THE PREPARATION OF VARIOUS TEAR-CONTAINING CREAMS AND INVESTIGATION OF THEIR ANTIBACTERIAL PROPERTIES

## Gözyaşı İçeren Farklı Kremlerin Hazırlanması ve Antibakteriyel Özelliklerinin İncelenmesi

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## ABSTRACT

In our research, the antibacterial properties of tear which protect our eyes from many infections have been investigated by using *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus* and *Staphylococcus epidermidis* bacteria. In addition, the antibacterial effect of creams in different formulations containing vaseline, also known as petroleum jelly, prepared by mixing olive oil and lemon juice which have been known and used throughout history with tears have been investigated. The antibacterial effect has been investigated measuring the zone of inhibition diameters, also known as the Kirby-Bauer method. It has been determined that all samples showed antibacterial effects except *Staphylococcus epidermidis* to which only tear was applied. The highest antibacterial activity with tear containing cream (vaseline and olive oil) has been observed for *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* bacteria. In addition, tear containing cream (vaseline, olive oil and lemon juice) has shown the highest antibacterial effect against *Bacillus subtilis* and *Staphylococcus haemolyticus* bacteria.

**Keywords:** Antibacterial activity, Olive oil, Tear, Vaseline, Zone of inhibition.

## ÖZ

Çalışmamızda, birçok enfeksiyondan gözlerimizi koruyan gözyaşının antibakteriyel özelliği *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus* ve *Staphylococcus epidermidis* bakterileri kullanılarak incelenmiştir. Ayrıca, antibakteriyel etkisi tarih boyunca bilinip kullanılan zeytinyağı ve limon suyunun, gözyaşı ile karıştırılmasıyla hazırlanan petrol jelatini olarak da bilinen vazelin içeren farklı formülasyondaki kremlerin antibakteriyel etkisi araştırılmıştır. Kirby-Bauer yöntemi olarak da bilinen inhibisyon zon çapları ölçülerek antibakteriyel etki araştırılmıştır. Sadece gözyaşı uygulanan *Staphylococcus epidermidis* hariç tüm örneklerin antibakteriyel etki gösterdikleri belirlenmiştir. En yüksek antibakteriyel aktivite, gözyaşı içeren krem (vazelin ve zeytinyağı) ile *Pseudomonas aeruginosa* ve *Staphylococcus epidermidis* bakterileri için gözlenmiştir. Ek olarak, *Bacillus subtilis* ve *Staphylococcus haemolyticus* bakterilerine karşı en yüksek antibakteriyel etkiyi gözyaşı içeren krem (vazelin, zeytinyağı ve limon suyu) göstermiştir.

**Anahtar kelimeler**: Antibakteriyel aktivite, Gözyaşı, İnhibisyon zon çapı, Vazelin, Zeytinyağı.

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## INTRODUCTION

Tear is an important secretion for the functioning of the eyes. The production system of tears consists of three parts. These are; the lacrimal gland where tear is produced, the channels that carry tear to the eyes and nose, and the lacrimal nucleus which is responsible for the connection of the nervous system. There are three different types of tears on the eye. These are called basic tear, reflex tear, and emotional tear (Farandos, Yetisen, Monteiro, Lowe, & Yun, 2015; Frey, Desota-Johnson, Hoffman, & McCall, 1981; Van Delft, Meijer, Van Best, & Van Haeringen, 1997).

Basic tear is responsible for the daily care of the eye. Living organisms other than humans can also produce it. Basic tear is produced on an average of 1.5 mL per day. This type of tear keeps the eye constantly wet (Goto et al., 2006; Toda, Fujishima, & Tsubota, 1993; Wolff, 1946). The functions of basic tear are: to obtain a smooth appearance, to remove substances (dust, hair, etc.) that may cause reflexes in the eye, to nourish the glassy structure called the cornea, to provide dissolved oxygen in the secretion, to protect the eye with included antimicrobial agents (antibacterials such as S-IgA, iso-aglutinin, lysozyme, lactoferrin, protease inhibitors) from pathogens that may cause infections. and to provide easy blinking by lubricating eyelids and creating an oily layer between eye and eyelids (Perkins & Davson, 2020). The basic tear has three different layers on the eye which differ from each other by function and structure. They are divided into three as mucin layer, aqueous layer, and lipid layer (Moshirfar et al., 2014; Lu et al., 2008). The mucin layer is secreted from goblet cells in the conjunctiva. By making the hydrophobic corneal surface into hydrophilic, it ensures homogeneous distribution of the aqueous layer on the entire eye surface. The aqueous layer is secreted by the main lacrimal gland, accessory glands, Krause and Wolfring glands. The accessory glands are located in the conjunctiva and are responsible for basal secretion. The functions of the aqueous layer are; to provide oxygen to the avascular corneal epithelium, to work as an antibacterial agent, to close small irregularities on the cornea, and to remove foreign bodies from the cornea. The lipid layer is secreted from the meibomian glands. These glands are located in the upper and lower eyelid canals and are emptied into the eyelashes. The functions of the lipid layer are; to create a hydrophobic barrier by increasing the surface tension, to provide great benefits in the prevention of eye dryness by delaying evaporation, and to help blinking comfortably by reducing the friction between eyelids and globe (sphere) thanks to its' oily structure (Perkins & Davson, 2020).

When the eye is faced with a physical, chemical or optical stimulus, it produces reflex tear to protect the eye and removes these stimuli from the eye to avoid eye irritation. The most well-known example of reflex tear is during chopping onions. Reflex tear is produced at a minimum of 100 µL per minute in case of any danger. While the potassium ($K^+$) concentration in the tear decreases in such a stimulation, the sodium ($Na^+$) concentration increases. The characteristic functions of reflex tear are; to protect the eye from cold, to repair the mechanical damage, to protect the eye from sharp odors as well as harmful chemicals (Moshirfar et al., 2014; Perkins & Davson, 2020).

The emotional tear, observed only in humans, is a secretomotor reaction that occurs with the secretion of fluid from the tear glands with the change of facial muscles. Emotional tear occurs with instinctive voices and in some moods with hiccups. The emotional tear production is at least 400 µL per minute, and it does not have a surface numbing task as reflex tear. The emotional tear has 24% more protein than the reflex tear. In addition, it has been determined that women have higher levels of prolactin, manganese, potassium, and serotonin compared to men in general (Frey et al., 1981).

Lysozyme plays the most important role in the prevention of bacterial infections in tear. Lysozyme (EC 3.2.1.17) is a glycoside hydrolase type enzyme found in body secretions such as saliva, tear, sweat, and mucus (Bron, Tiffany, Gouveia, Yokoi, & Voon, 2004; Goto et al., 2006; Nagyova & Tiffany, 1999). In the studies of Zahoor et al., the effectiveness of tear on the bacteria was observed (Zahoor, Bahadar, Ayaz, Khan, & Shah, 2018). Apart from lysozyme, there are various lactoferrin, antibodies, and more than 400 antibacterial agents in tear (Arnold, Brewer, & Gauthier, 1980).

Tear is simply composed of water, salt, antibodies and enzymes that have both antimicrobial and immunological activity. This gives a bactericidal feature to tear (this is the basis of the bactericidal feature in tear). Since the antibacterial activity of lysozyme is well known, it is used in the food industry to prevent food spoilage (Brasca et al., 2013; Durance, 1994; Tirelli & De Noni, 2007). Previous research published by Friedlland et al. shows that non-lysozyme factors are mostly responsible for the bactericidal features, while lysozyme is responsible for only 0.5% antibacterial activity of tear (Friedland, Anderson, & Forster, 1972). However, in the studies conducted by Seal et al., they reported that when the anti-lysozyme agent was added to the tear, the antibacterial activities of the tear were eliminated. It had been reported that lysozyme has antiviral activity since it corresponds to 30% of tear proteins and has been identified as the main antibacterial component of tear fluid (Seal, Mackie, Coakes, & Farooqi, 1980). Lysozyme (EC 3.2.1.17) is the most researched antimicrobial enzyme. Besides,

lysozyme is abundant in a few mammalian secretions such as milk, saliva, and tear. It is a low molecular weight (14,307 Daltons) enzyme composed of 129 amino acids cross-linked by four disulfide bridges. Lysozyme has lytic activity on the β (1→4) glycosidic bond (Bron et al., 2004; Silvetti et al., 2010; Van der Strate, Beljaars, Molema, Harmsen, & Meijer, 2001). However, there are a few researches related to the antimicrobial activity of tear in the literature, the antibacterial properties of different cream formulations consisting of tear have not been studied before.

In this study, the zone of inhibition method, also known as the Kirby-Bauer method, was used. This commonly used method is a live-material susceptibility test which the material is left on the solid medium over time. Then, it shows its antimicrobial effect on a field called the zone of inhibition (Başustaoğlu, Yıldırım, & İnce, 2020). Antibacterial and antifungal studies are frequently performed with this method, and the results can be easily observed on petri dishes (Poupard, Rittenhouse, & Walsh, 1994). The purpose of this article is to observe the antibacterial effects of creams having a variety of contents, which is expected to change over different types of bacteria with the addition of tear, using the zone of inhibition method.

## MATERIAL AND METHOD

### Chemical Materials

Luria Bertani (LB) broth as a nutrient supplement for bacteria was purchased from Neogen (Lansing, MI/USA). Agar, which is used as the solidifying agent, was bought from the company named Liofilchem (Italy). The brand name of vaseline, which is used as fundamental component for the cream formulation in mixture, was Saran (Konya, Turkey). The vaseline, which consists of pure petroleum jelly, amounts of all samples except only tear without cream were constant as 1.8 grams.

### Sample Preparation Procedures

For the method, our samples were classified into 6 groups as shown on below Table 1.

**Table 1.** The Categorization of Samples

| Groups | Samples |
|---|---|
| 1 | Only Tear |
| 2 | Cream* (Vaseline) |
| 3 | Cream* (Vaseline) + Tear |
| 4 | Cream* (Vaseline + Olive Oil) |
| 5 | Cream* (Vaseline + Olive Oil) + Tear |
| 6 | Cream* (Vaseline + Olive Oil + Lemon Juice) + Tear |

*All of the cream samples have glycerol in order to give them accurate cream forms.

**The Preparation** of **Only Tear without Cream**

In the first stage, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis* bacteria were grown in Luria Bertani (LB) broth at 37°C overnight. All of the tear samples in this research were obtained from a girl. It was observed that her emotional tears were aseptically accumulated in glass tubes. Then, only 10 µL drops of tear without any cream were dropped on filter papers with a diameter of 6 mm, and the discs to be placed on petri dishes were prepared by waiting for them to dry. Solid media containing LB/Agar were prepared and autoclaved and poured into each petri dish as 15 mL under aseptic conditions and allowed to solidify. After solidification, 100 µL of each type of bacteria was spread into a petri dish and the experiment was repeated 3 times. Petri dishes were kept at 37°C overnight, and the following data were obtained by measuring the zone diameters of the bacteria-free region (the region where bacteria died) because of only tear in the area where the discs were placed the next day.

**The Preparations of Creams Having Only Vaseline, Vaseline with Olive Oil without Tear**

In the next step, experiments with only vaseline and vaseline with olive oil creams without added tear were designed. For these experiments, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis* bacteria were grown in Luria Bertani (LB) broth at 37°C overnight. Solid media containing LB/Agar were prepared and autoclaved and poured into each petri dish as 15 mL under aseptic conditions and allowed to solidify. After solidification, 100 µL of each bacterial species was spread into a petri dish.

In the preparation of only vaseline and vaseline with olive oil creams, 300 µL of glycerol was added to the cream containing only 1.8 grams of vaseline for no tear added. Afterwards, 1.8 grams of creams (only vaseline and vaseline + olive oil) were mixed until homogeneous. Then, both types of cream were divided into 6 parts, each of which was 0.3 grams. Every cream was shaped into a cylindrical shape and 6 mm diameter wells were drilled into each LB/Agar-containing solid media and these only vaseline and vaseline + olive oil creams were filled into the wells (Figure 1). Petri dishes were kept at 37°C overnight, and the next day, the zone diameters of the bacteria-free areas (the area where the bacteria died) were measured in the areas where the tearless creams were placed.

## The Preparation of Cream Having Vaseline with Olive Oil plus Tear

In the next step, the experiment, vaseline with olive oil cream plus tear, was done. For this experiment, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis* bacteria were grown in Luria Bertani (LB) broth at 37°C overnight. Nutritionally rich medium containing LB/Agar was prepared, and autoclaved. After this procedure, the medium was poured into each petri dish as 15 mL under aseptic conditions and allowed to solidify. After solidification, 100 µL of each bacterial species was spread into a petri dish.

In the preparation of vaseline with olive oil cream, 300 µL of glycerol was added to the cream containing only 1.8 grams of vaseline for tear added. For the tear additive, 300 µL of glycerol was added to 300 µL of tear as 1:1 ratio. Afterwards, 1.8 grams of cream (vaseline + olive oil) were mixed until homogeneous. Then, the prepared cream was divided into 6 equal weights of 0.3 grams each. Every cream was shaped into a cylindrical shape and 6 mm diameter wells were drilled into each LB/Agar-containing solid media and this vaseline + olive oil cream was filled into the wells (Figure 1). Petri dishes were kept at 37°C overnight, and the next day, the zone diameters of the bacteria-free areas (the area where the bacteria died) were measured in the areas where the cream with tear was placed.

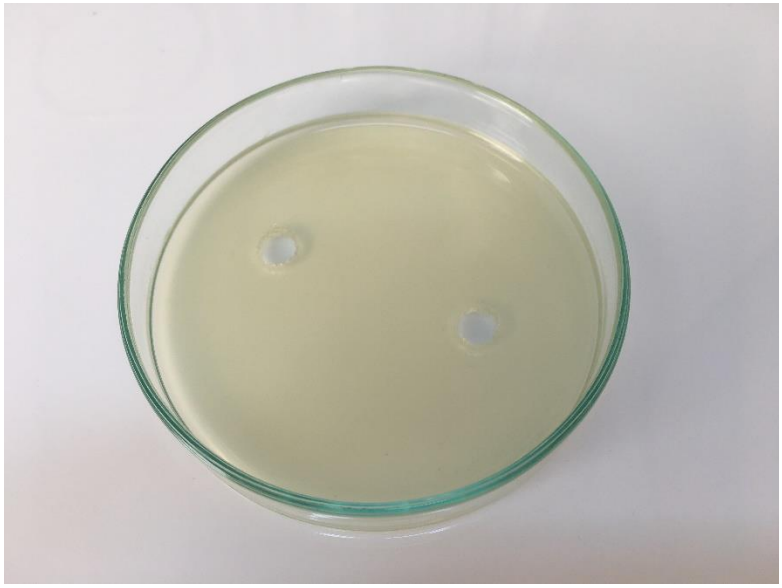

**Figure 1.** LB/Agar-Containing Solid Media Having 6 mm Diameter Wells in a Petri Dish

## The Preparation of Cream Having Vaseline with Olive Oil and Lemon Juice plus Tear

As the next step, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis* bacteria were grown in Luria Bertani (LB) broth at 37°C overnight. Solid media containing LB/Agar were prepared and autoclaved and poured

into each petri dish as 15 mL under aseptic conditions and allowed to solidify. After solidification, 100 µL of each bacterial variety was spread into a petri dish.

In the preparation of the cream, 300 µL of glycerol was added to 300 µL tear in a 1:1 ratio. Afterwards, it was mixed with 1.8 grams of cream (containing lemon juice, vaseline, and olive oil) until homogeneous. Then, it was divided into 6 parts weighing 0.3 grams. Each piece was cylindrical shaped and 6 mm diameter wells were opened in the middle of each LB/Agar-containing solid media and these creams were filled into the created wells. Petri dishes were kept at 37°C overnight, and the next day, the zone diameters of the bacteria-free zone (the zone where the bacteria died) were measured because of the creamy tear formed in the area where the creamy tear were placed.

### Ethical Considerations

Approval from The Ethics Committee of Inonu University, Malatya Clinical Researches (2021/152) was obtained.

## RESULTS AND DISCUSSION

### The Experiment of Only Tear (without Cream)

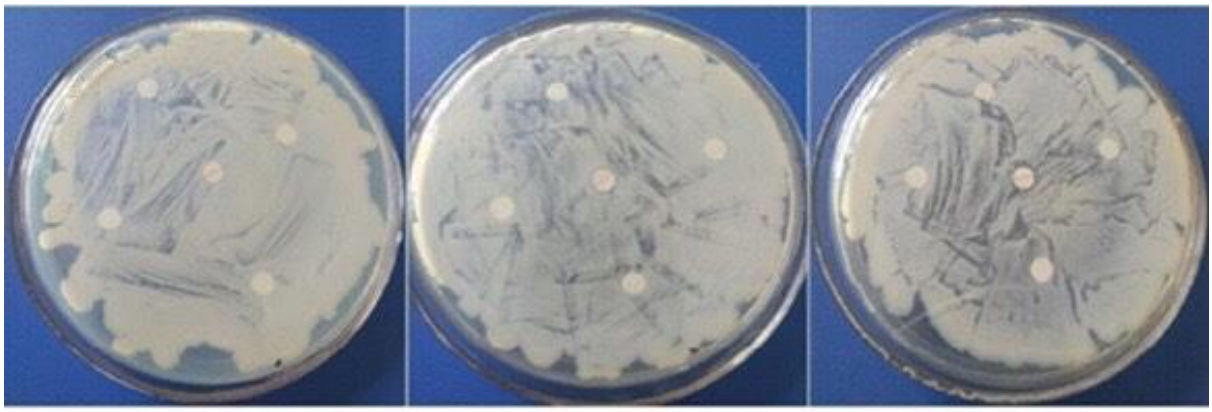


**Figure 2.** The Antibacterial Effect of Only Tear (without Cream) on *Bacillus Subtilis*


**Figure 3.** The Antibacterial Effect of Only Tear (without Cream) on *Pseudomonas Aeruginosa*

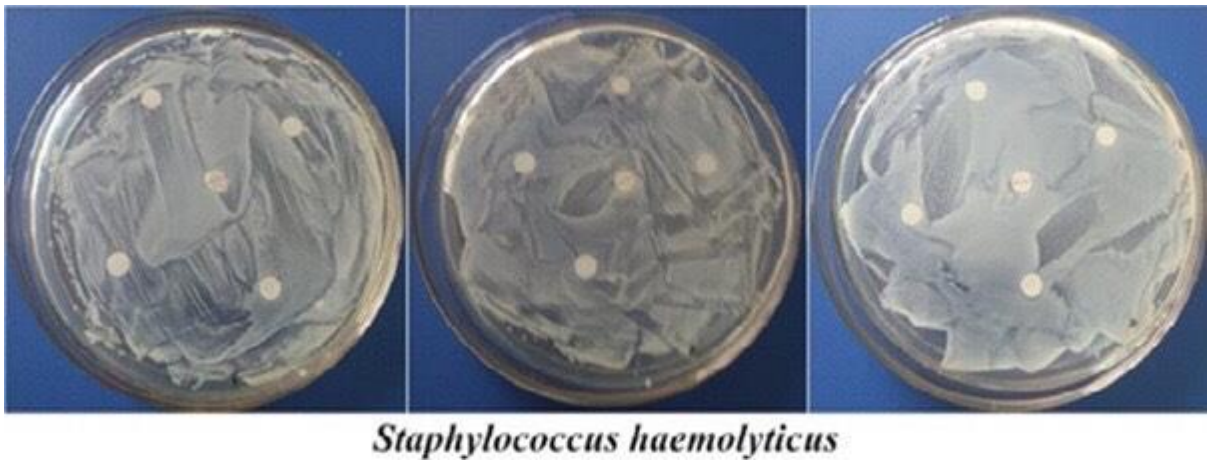


**Figure 4.** The Antibacterial Effect of Only Tear (without Cream) on *Staphylococcus Haemolyticus*

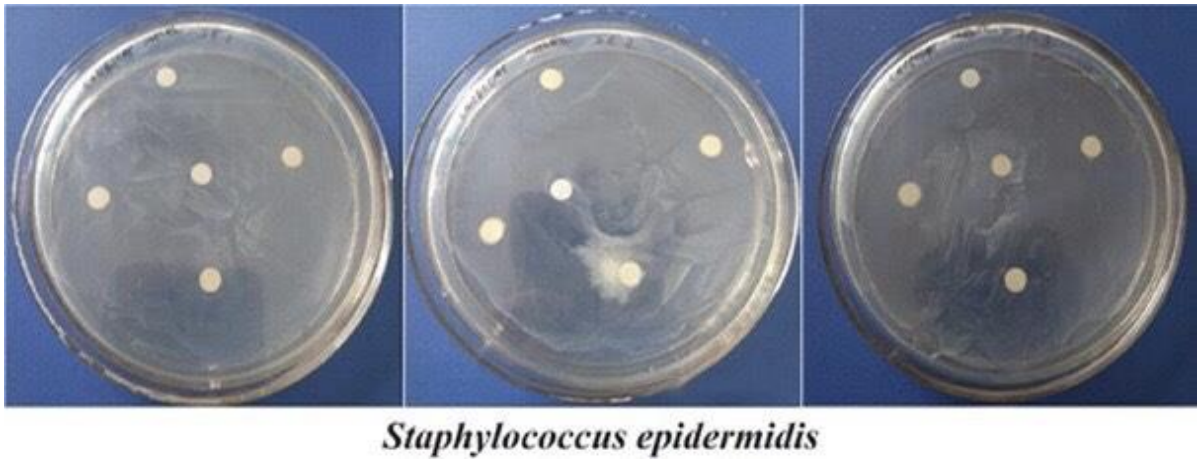


**Figure 5.** The Antibacterial Effect of Only Tear (without Cream) on *Staphylococcus Epidermidis*

As can be seen from the zone diameter photographs on figure 2, 3, 4, and 5 above, no zone diameter was observed in *Staphylococcus epidermidis* bacteria, while zone diameters were observed in *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus haemolyticus* bacteria. When the zone diameters were compared, it was observed that only tear was effective in *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus haemolyticus* bacteria, respectively. When our only tear experiment results are compared to the literature in children,

it is obvious that all of the results in terms of antibacterial activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus haemolyticus* bacteria except *Staphylococcus epidermidis* are higher than *Escherichia coli*, *Staphylococcus aureus*, *Shigella sonnei*, and *Salmonella typhi* bacteria (Friedland et al., 1972; Seal et al., 1980; Zahoor et al., 2018).

## The Experiments of Creams Having Only Vaseline and Vaseline plus Olive Oil (with and without Tear)

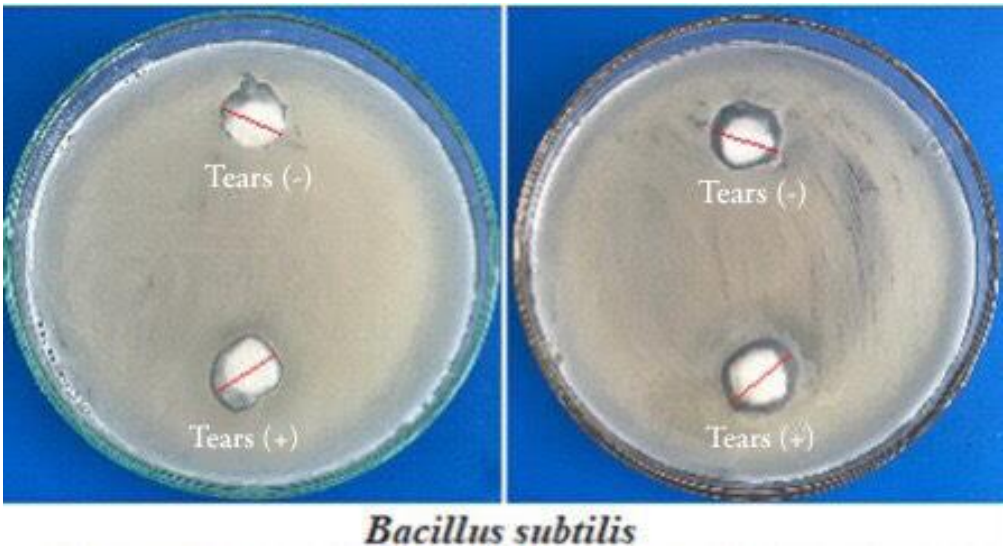


**Figure 6.** The Antibacterial Effect of Creams Having Only Vaseline and Vaseline with Olive Oil (with (+)/without (-) Tear) on *Bacillus Subtilis*


**Figure 7.** The Antibacterial Effect of Creams Having Only Vaseline and Vaseline with Olive Oil (with (+)/without (-) Tear) on *Pseudomonas Aeruginosa*

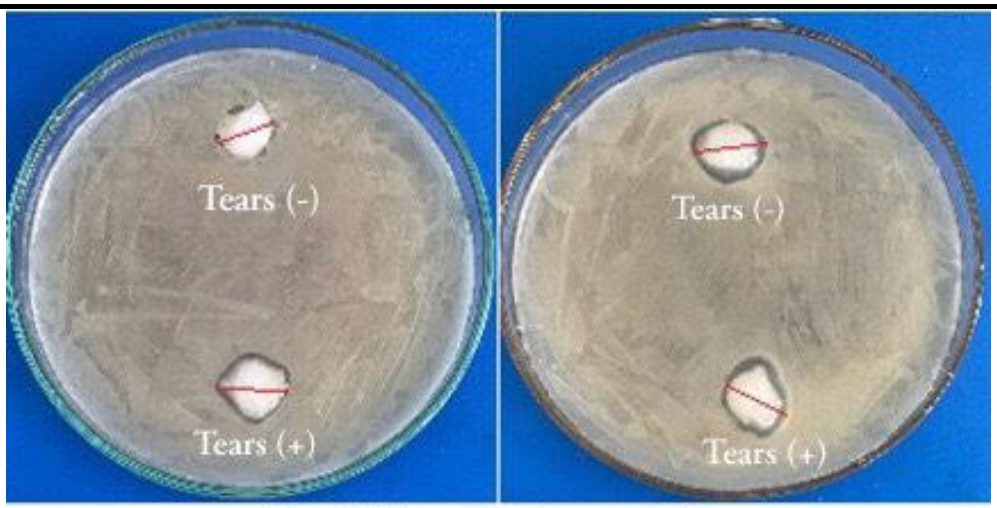


**Figure 8.** The Antibacterial Effect of Creams Having Only Vaseline and Vaseline with Olive Oil (with (+)/without (-) Tear) on *Staphylococcus Haemolyticus*


**Figure 9.** The Antibacterial Effect of Creams Having Only Vaseline and Vaseline with Olive Oil (with (+)/without (-) Tear) on *Staphylococcus Epidermidis*

As can be seen from the zone diameter photographs on figure 6, 7, 8, and 9 above, zone diameters were seen in all bacteria. When the zone diameters were compared, it was understood that both creams containing tear (vaseline only and vaseline + olive oil) were more effective than creams without tear. In addition, according to the zone diameters of the creams containing only vaseline and vaseline + olive oil, vaseline plus olive oil sample was more effective against all bacterial species than the only vaseline containing cream, whether or not it contains tear.

**The Experiment of Cream Having Vaseline + Olive Oil + Lemon Juice with Tear**

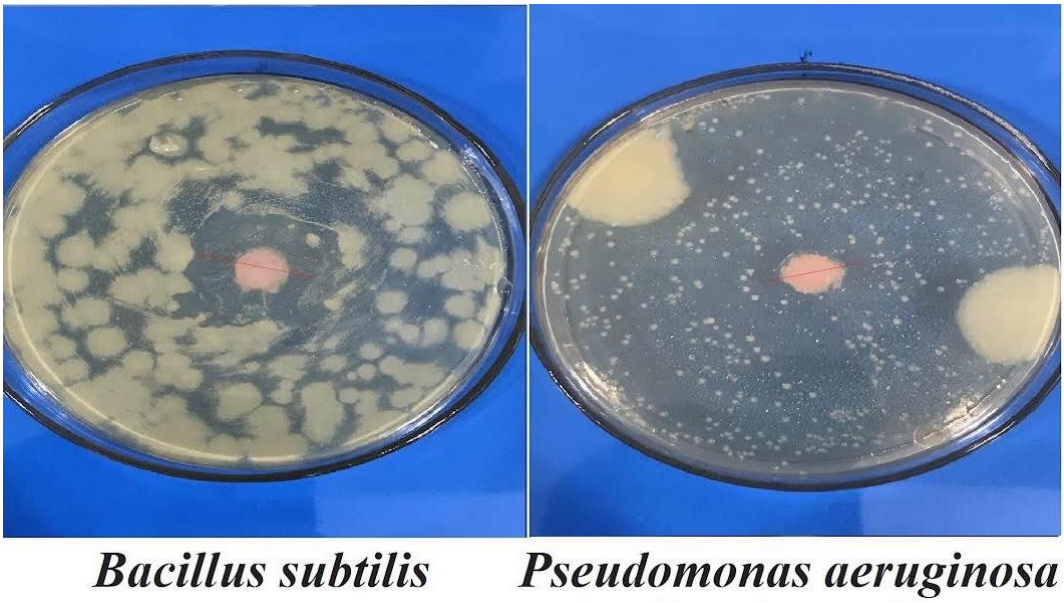


**Figure 10.** The Antibacterial Effect of Creams Having Vaseline + Olive Oil + Lemon Juice with Tear on *Bacillus Subtilis* and *Pseudomonas Aeruginosa*

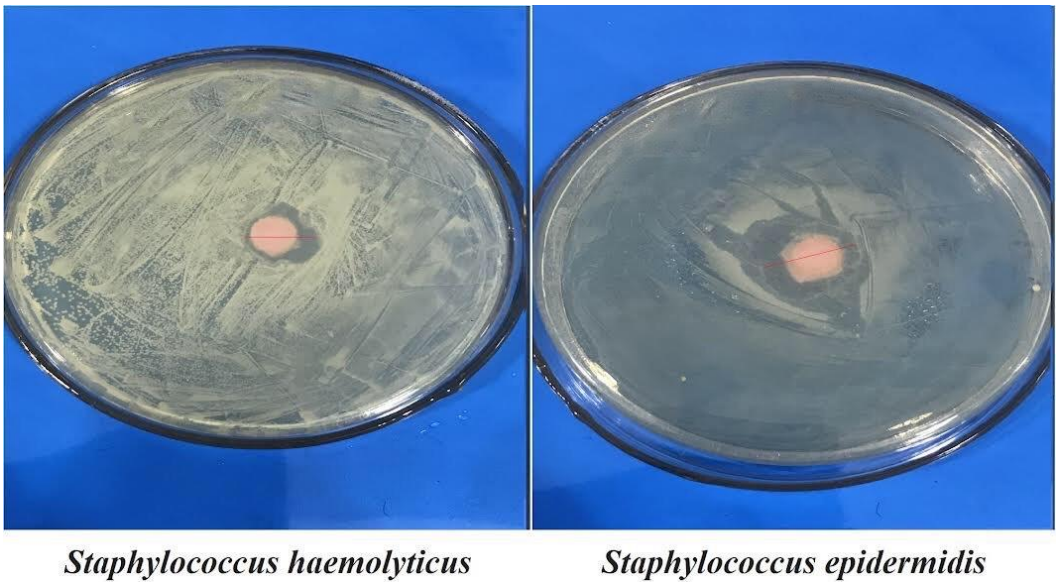


**Figure 11.** The Antibacterial Effect of Creams Having Vaseline + Olive Oil + Lemon Juice with Tear on *Staphylococcus Haemolyticus* and *Staphylococcus Epidermidis*

As can be seen from the zone diameter photographs on figure 10, and 11 above, zone diameters were seen in all *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis* bacteria. When the zone diameters were compared, it was observed that creamy tear containing vaseline + olive oil and lemon juice were most effective on *Bacillus subtilis* bacteria, followed by *Staphylococcus epidermidis*, Sta*phylococcus haemolyticus*, and *Pseudomonas aeruginosa* bacteria, respectively.

Zone of inhibition diameters of *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis* bacteria related to our research can be seen on Table 2.

**Table 2.** Zone of Inhibition Diameters (mm)

| **Samples** | **Zone of Inhibition Diameters (mm)** | | | |
|---|---|---|---|---|
| | *Bacillus subtilis* | *Pseudomonas aeruginosa* | *Staphylococcus haemolyticus* | *Staphylococcus epidermidis* |
| Only Tear | 7.33 ± 1.53 | 7.67 ± 0.58 | 6.33 ± 0.58 | 0 |
| Cream (Vaseline) | 13.33 | 12 | 8.33 | 9.33 |
| Cream (Vaseline) + Tear | 14 | 15 | 11.66 | 12 |
| Cream (Vaseline + Olive Oil) | 15.33 | 16.33 | 14.33 | 16.66 |
| Cream (Vaseline + Olive Oil) + Tear | 17 | 20.33 | 14.66 | 22.66 |
| Cream (Vaseline + Olive Oil + Lemon Juice) + Tear | 24 | ~19 | 19 | 20 |

## CONCLUSION

In a nutshell, the increased antibacterial activities were seen as a consequence of diversifying the cream content in order of only vaseline, vaseline + olive oil, and vaseline + olive oil + lemon juice. Furthermore, the maximum zone of inhibition diameters were obtained with the addition of tear into these creams. The antibacterial effects of all samples were determined except the only tear sample over *Staphylococcus epidermidis*. The highest antibacterial activities were observed with cream (vaseline + olive oil) + tear for *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* bacteria. Besides, cream (vaseline, olive oil, and lemon juice) plus tear showed the most antibacterial effects against *Bacillus subtilis* and *Staphylococcus haemolyticus* bacteria, respectively.

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