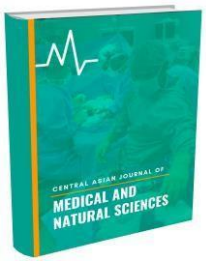

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Article

Evaluation of Aloe Vera Ethanolic Extract and Medical Al-Athmad Eyeliner on Bacterial Species Causing Eye Inflammation

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Abstract: The current study included the collection of (300) samples from (150) people from patients visiting Balad General Hospital and some private clinics in Balad District - Salah Al-Din Governorate, a sample was taken from both the right and left eyes using special dry and sterile cotton swabs that can be disposed of later, for the period ranging from November 2022 to January 2023, (235) positive samples for bacterial growth were obtained, constituting 78.3% of the total samples collected, positive growth specimens have been diagnosed depending on their morphological, farming and biochemical properties, the diagnosis of a number of them was confirmed using the Vitek2 system. The results showed obtaining (140) isolates out of a total of (235) positive samples for Gram stain, with a percentage of 59.6%, it included aureus staphylococcus by 27.65%, Staphylococcus epidermidis by 14.89%, Bacillus by 6.36%, Streptococcus viridans by 4.25% and Diphtheria by 6.38%, while the negative isolates of Gram stain were (95) isolates with a percentage of 40.4% of the total isolates and included Psedomonas auroginosa by 14.89%, klebsilla bacteria by 8.51%, E-Coli bacteria by 10.63% and Nisseria gonorrhoeae bacteria by 6.38%. The results also showed that the eyeliner used in the study (Al-Athmad) gave a modest inhibitory activity compared to the ethanolic alcoholic extract against the bacterial species under study, the highest average inhibitory diameter was (15) mm for N. gonorrhoeae and S. viridans at concentration (200) mg/ml.

Keywords: Extracts, Antimony kohl, Aloe vera, bacterial inhibition, ophthalmitis

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1. Introduction

The eye is one of the most vulnerable organs of the body to various environmental factors, so there are multiple defense factors that reduce the incidence of microbes that reside on the surface of the eye [1], the tear also works with the movement of the eyelids to expel foreign substances out of the eye, as well as the presence of lysozyme, lactoferrin, secretory antibodies (IgA), Defensives in high concentrations in the tear [2], and the natural flora is found in the conjunctiva of the eye [3], which works to produce (bacteriocins) and other inhibitory substances such as lactico and acetic acids that prevent the invasion of pathogenic microorganisms [4]. However, there are many factors that enable pathogenic microorganisms to enter the eye and infect it through the use of contaminated materials and tools during eye surgeries and transmission of bacteria from areas near the eye and

prolonged use of antibiotics affecting the normal flora of the eye [5], it helps the emergence of antibiotic-resistant strains of bacteria and fungi [6].

One of these cosmetic products used is eyeliner, as pure eyeliner (Al-Athmad) contains antimony sulfide and trisulfide as two main components, they have a therapeutic effect on some eye conditions caused by bacteria [7], including conjunctivitis [8]. From the above, it is clear that the study of the inhibitory activity of Aloe Vera L [9]. As a solution to the medical antimony for the growth of pathogenic bacteria negative and positive for Gram stain because they contain many compounds anti-bacterial growth hence the idea of this study, which aimed to:

1. Isolation and diagnosis of some bacterial species that cause eye inflammation from patients attending Balad General Hospital and some private clinics.
2. Determination of the biological efficacy of both medical eyeliner and Aloe vera L. ethanol extract against various bacterial etiology.

2. Materials and Methods

Preparation of Culture Media:

The ready-made planting media were prepared according to the instructions of the manufacturers and fixed on the packaging of each medium, the pH was adjusted to 7.0 and sterilized according to the type of culture medium at 121°C and under pressure of 15 lb/ang2 for 15 minutes, after that, the planting media was incubated after pouring them into the dishes or tubes according to the requirements of the experiment at 37 m for 24 hours to ensure that there was no contamination, then kept in the refrigerator at a temperature of 4 ° C until use and attended the media according to what was stated in [10].

Bacterial Culture of Samples:

Bacterial culture of incubated samples with nutrient broth in the laboratory, where three media were prepared for bacterial culture, namely the middle of blood agar, the center of the macConki agar and the medium of saline mannitol, and then part of the incubated sample was transferred with nutritious broth using a flame-sterilized germ carrier, by filling the conveyor loop to be planted on the dishes containing the growing media, then all the dishes were incubated in the incubator at 37 pm for 24 hours, the developing colonies were then examined and the required isolation and diagnostic tests were performed.

Plant Collection and Sample Preparation:

Aloe vera leaves of 30-60 cm in lengths were collected from a group of nurseries belonging to the city of Balad / Salah Al-Din, the plant was classified by Dr. Omar Khalil Jassim Al-Abbasi, a plant classification specialization in the research and development department of the State Company for Pharmaceutical Industry / Samarra, where the leaves were cleaned and washed with distilled water several times to clean them of dust and dust, they were then left to air dry, left in the shade for several days until dry and then cut into small pieces to be ready for extraction.

Alcoholic Extraction:

The ethanolic alcoholic solvent was used in the preparation of the extract, where 100 g of dried and cut aloe leaves were mixed in a glass flask with 500 ml of the specified solvent, leave the mixture at room temperature for 72 hours, shaking occasionally and periodically, then the mixture was filtered using pieces of sterile gauze and then using Whatman filter paper, then take the filter and evaporate the rotary evaporator at a temperature of 55 ° C until the solvent is completely disposed of, the remaining material was then placed in airtight glass containers in the refrigerator until use [11].

Sterilization of Alcoholic Extracts of the Aloe Vera Plant:

Prepare the alcoholic extract by dissolving 1 g of dry alcoholic extract in 10ml of distilled water to obtain a concentration of 200%, then the mixture was sterilized by

pasteurization method at a temperature of 62.8 for 30 minutes, this obtained the standard concentration that was used to obtain the rest of the fear.

Statistical Analysis

SAS (2004) was used in statistical analysis of data to study the effect of antibiotics, plant extracts and studied treatment concentrations on inhibition ratio, significant differences between averages were compared with the least significant difference (LCD) test, the chi-square test (x2 – Chi – Square) was also used to compare the significant differences between the studied ratios [12].

3. Results and Discussion

Sample Collection and Isolation

The study included the collection of 300 samples from 150 patients visiting Balad General Hospital, and some private clinics from both the right and left eyes using special sterile dry cotton swabs that can be disposed of for the period ranging from November 2022 to January 2023, the patients underwent a clinical examination by a specialist doctor and the samples were collected using sterile cotton swabs containing a transport media implant medium, as they were rotated on the part to be taken from the sample and these swabs were transferred to the microbiology laboratory for the purpose of implantation and diagnosis, where the middle of blood and MacConkey agar and the medium of chocolate agar was used to develop and isolate bacteria and was incubated at a temperature of 37 °C for 24 hours.

The results of the initial transplantation of 300 samples showed that 235 of them gave bacterial growth of 78.3%, while 65 samples did not give bacterial growth of 21.7% (Table and Figure 1), this unipositive ratio of culture growth may be attributed to patients taking antibiotics that enhanced the body's immune defense ratio [13] or the reason for the failure to grow may be due to the fact that the pathogen is viral, fungal, parasitic or anaerobic bacteria, which are difficult to isolate in the same ways used to isolate aerobic bacteria because they need special environments and conditions for development [14].

Table 1. Results of sampling

Transplant results	Number	Percentage (%)
Positive growth	235	78.3%
Negative growth	65	21.7%
Total	300	100 %

This study was close to the findings of the researcher [15]. as the positive result of growth was 70%, our results were similar to those of the researcher (Hus et. al.,2024) where the positive rate of bacterial culture of samples taken from the eye was 77%, it was lower than that obtained [16]. with 80% positive growth in samples taken from the eyes of healthy people using contact lenses [17].

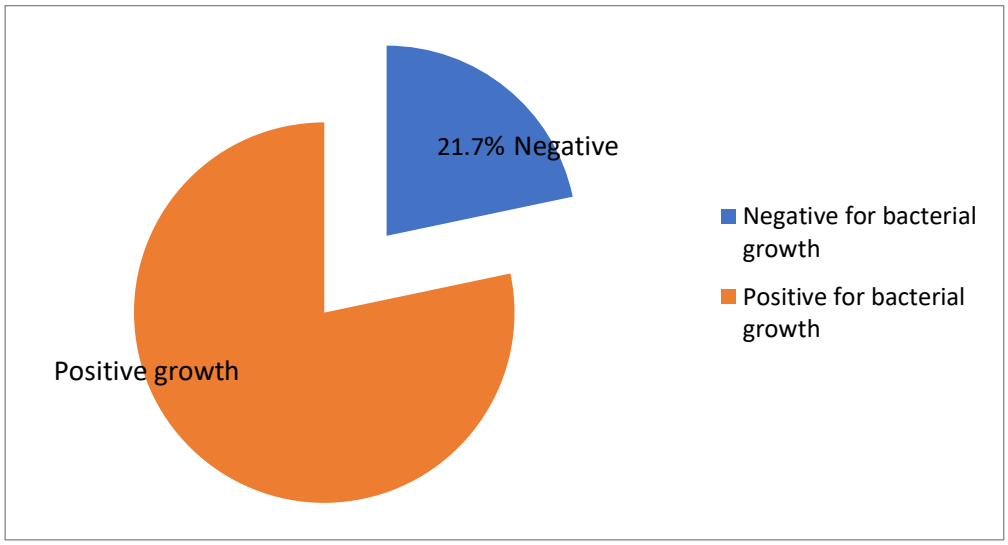

Figure 1. Shows the percentages of eye injuries

The emergence of infection is an infectious disease that is easily transmitted as the infection arises directly from contact with the contaminated hand with the eye, or from exposure to polluted air, or as a result of the spread of the pathogen with respiratory secretions and thus cause infection, also [18], sometimes the conjunctiva may colonize some types of bacteria from the nasopharynx as a result of blockage of the tear duct and nose, or infection may occur when exchanging tools with infected people [19].

Test Of Inhibitory Efficacy of Aloe Vera Ethanolic Extract:

The results showed that the ethanolic aloe vera extract had a strong inhibitory effect on bacterial growth, as the statistical analysis between the averages of the concentrations used showed that there is no significant difference between the two concentrations (50, 25), it has a greater effect on S. aureus bacteria, with an average inhibition diameter of all concentrations of (14.6) mm. Table 2.

Table 2. Effect of Aloe Vera Plant Ethanolic Extract Concentrations on Isolates Under Study

Type of bacteria	Concentration in milligrams					Average effect of extract concentrations
	1	**2**	**3**	**4**	**5**	
	25	**50**	**75**	**100**	**200**	
S.aureus	11	14	15	16	17	14.6 A
S.epidermides	7	9	12	14	15	11.4B
Klibsiella	6	6	15	17	26	14.0A
E.coli	0	0	4	12	15	6.2 D
Bacillus	10	10	10	15	15	12.0B
N. gon.	0	0	6	10	20	7.2CD
Diphtheria	10	10	11	18	21	14.0A
Pseudomonas	5	6	10	11	23	11.0B
S.viridans	0	5	10	10	15	8.0C
Average concentration	**5.4 d**	**6.7 d**	**10.3 c**	**13.7 b**	**18.6 a**	

Small letters that are similar horizontally mean that there is no significant difference between them.

Vertically similar capital letters mean there is no significant difference between them.

The importance of aloe vera in the fight against microbial infections is shown, and this plant is also used in folk medicine to treat various diseases, and pay attention to the

importance of the aloe vera plant and its selection in further research and the discovery of new bioactive compounds. The result of this study showed that the ethanol extract of aloe vera has good antimicrobial properties and even shows increased inhibition diameters with increased extract concentrations [20]. This study shows that we can use aloe vera as an antimicrobial agent in new drugs to treat infectious diseases in humans [21]. The results of this research have been developed specifically for a variety of Gram-negative pathogen bacteria and Gram-positive pathogens, inhibitory isolation compounds have clearly proven their usefulness for various pathogenic microorganisms [22], and explains that the application of plants in traditional medicine in the treatment of various diseases caused by these pathogenic strains. Plants are used in traditional medicine, as well as plants are used in the treatment of various diseases of these pathogenic strains. Furthermore, the identification of these antimicrobial compounds promotes their growth through the study of the structure/activity of new antimicrobials [23].

Testing the Inhibitory Effectiveness of Eyeliner

The results showed that eyeliner had a moderate inhibitory effect on bacterial growth, where the statistical analysis between the averages of the concentrations used showed high variation significantly, the inhibitory effect of eyeliner against bacterial species under study increases as the concentration of eyeliner increases, as we note in the table below the highest inhibition of most isolates in the fifth concentration of 200 mg Table (3).

Table 3. Effect of Eyeliner concentrations on isolates under study

Type of bacteria	Concentration in milligrams					Average effect of extract concentrations
	1	**2**	**3**	**4**	**5**	
	25	**50**	**75**	**100**	**200**	
S.aureus	**10**	**10**	**11**	**14**	**14**	11.8 A
S.epidermides	**10**	**10**	**11**	**11**	**14**	11.2 B
Klibsiella	**2**	**10**	**11**	**12**	**13**	9.6 C
E.coli	**4**	**7**	**9**	**12**	**12**	8.8 E
Bacillus	**6**	**10**	**10**	**11**	**13**	10.0 C
N. gon.	**0**	**0**	**10**	**11**	**15**	7.2 F
Diphtheria	**0**	**6**	**13**	**13**	**13**	9.0 D
Pseudomonas	**7**	**7**	**8**	**8**	**14**	8.8 E
S.viridans	**10**	**10**	**10**	**15**	**15**	12.0 A
Average concentration	**5.4 e**	**7.8 d**	**10.3 c**	**11.9 b**	**13.7 a**	

Small letters that are similar horizontally mean that there is no significant difference between them.

Vertically similar capital letters mean there is no significant difference between them.

Application of eyeliner in the formation of the eyes also thin layer with precorneal rupture film that prevents the lens and retina from UV rays, thus plays an important role in the prevention and treatment of eye diseases such as conjunctivitis, posterior blepharitis, delay formation of cataracts etc. Eyeliner is also said to be used to keep eyes clean and cool, sharpen vision and support them [24].

It is important for many patients with blepharitis to seek more effective substances to prevent pathogenic outcomes caused by bacterial infections, there are many conventional therapies proposed by researchers and studies, but due to the lack of a proven response to these treatments at the physiological level, they are not applied,

studies suggest further mechanisms to understand the etiology and associated factors to control blepharitis, one of the most common treatments for eyelid diseases in Persian medicine texts is the use of eyeliner. Eyeliner is a mineral substance of cold and dry nature, and according to Persian medicine, it has many benefits and traditional healers have paid special attention to the treatment of eye diseases, for example, applying eyeliner on the eyelid can prevent cataracts and eye ulcers, and according to Persian medicine, eyeliner strengthens the eye and maintains eye health, Ibn Sina claims in Gannon that eyeliner eliminates any type of infection and secretion caused by lesions inside the eye and maintains eye health [25].

One of the studies suggested that a culture medium containing eyeliner had an inhibitory effect on staphylococcal growth [26]. According to Al-Kaff et al., eyeliner has an inhibitory effect against staphylococcus [27]. Mahmoud et al. also noted that eyeliner led to an increase in nitric oxide production with antimicrobial properties [28]. According to Gupta researcher, eyeliner formulated has antimicrobial activity as the chemical composition of eyeliner can explain its antibacterial and anti-inflammatory properties, Chemical analysis of eyeliner indicates that the main elements of eyeliner include lead, sulfur, carbon, iron and zinc [29]. Based on studies, sulfur nanoparticles have bactericidal efficacy against many bacteria, including Staphylococcus aureus; furthermore, antimob has antibacterial activities [30].

4. Conclusion

The study demonstrates the significant antimicrobial properties of Aloe Vera ethanolic extract and the moderate inhibitory effect of medical eyeliner against various bacterial isolates. Aloe Vera shows potential as an antimicrobial agent in new drugs, while eyeliner can play a role in preventing and treating eye infections. Further research is recommended to explore new bioactive compounds in Aloe Vera and the full potential of eyeliner in traditional and modern medicine.

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