Academicia Globe:
Inderscience Research

ISSN: 2776-1010 Volume 4, Issue 1, Jan., 2023

IMPACT OF FENUGREEK PLANT EXTRACT ON THE NATURAL FLORA OF THE EYES OF CONTACT LENS AND EYELINER USERS

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Abstract

The study was conducted to isolate and diagnose normal flora in the eyes and comparing them with contact lens and eyeliner users. In addition, study the inhibitory ability of ten antibiotics and identifying the ability of the aqueous alcoholic plants extract of fenugreek to inhibit them biologically. The results of the bacterial culture showed that 112 samples being tested were positive bacterial growth out of 160 samples. The results of the isolation and diagnosis showed that the percentage of positive bacteria for gram stain was 70%.5 percent. The number of positive isolates of Gram stain was 79% and the largest percentage of these was the coagulase-negative staphylococci. The number of samples of this type was 72 of the total samples by 62%, whereas Gram-negative bacteria were 28 samples which meant 25%.

The results indicated that the largest number of bacteria belonged to the group of users of kohl and cosmetics by 37 isolates of gram-positive bacteria stain and 9 negative bacteria so the total number reached 46 isolates. As for the contact lenses, it was found that the number of gram-positive bacteria stain reached 27 isolates while the percentage of gram-negative isolates were 18 and the total number of isolates tested was 45. The control group who did not use eyeliner nor contact lenses, positive bacteria was 20 isolates whereas negative showed only one isolate. Hence, the total number was 21 isolates. It was found out that the least number and the least diverse of isolates were in the control group.

The results of the allergy test showed that all the isolates were resistant to antibiotic ceftazidime and all the isolates were sensitive to levofloxacin and ofloxacin. As for the rest of the antibiotics, the isolation resistance was uneven, the total resistance of the streptomycin was 58.9% whereas the total resistance of neomycin antibiotic was 42.8% and the antibody ratio of clindamycin was 38.3%. As for the resistance ratio of piperacillin was 78.5%, doxycycline antibiotic its resistance ratio was 37.5% and the resistance ration of chloramphenicol was 50.8%. Finally, the resistance ratio of erythromycin was 68.7%. The results revealed that the aqueous plant extracts for fenugreek were more efficient than it"s alcoholic extracts, the results showed that the greater the concentration was the greater the inhibitory capacity of the fenugreek would be.

Keywords: Natural flora, Fenugreek, S. warneri, Eyeliner users, E. coli, Plant extracts.

Introduction

The image that we see is formed through a complex optical system created by God to see the universe with this ease and smoothness, and it is exposed to many types of bacteria, fungi and parasites due to the use of cosmetics, lenses and eyeliner, medical or cosmetic lenses can lead to eye damage, as the

ISSN: 2776-1010 Volume 4, Issue 1, Jan., 2023

improper use of the lens and the pollution present in the person's hand or in the lens preservative solution, in addition to the poor quality of the lens itself, it leads to the multiplication of microorganisms in the eye, such as bacteria, fungi, and yeasts, and it has been proven that bacteria are the most common cause of infectious keratitis [i,ii].

The surface of the body supports the growth of a variety of bacteria and fungi that are collectively called the normal flora. Viruses and parasites are not natural flora, although they are present in asymptomatic individuals, these bacteria may be present and play a role that is normally important to bodily function and health because they prevent the establishment of invasive and pathogenic microorganisms[iii]. Normal flora play an important role in normal body function and health by secreting chemicals to maintain surface homeostasis and regulate immunity. They also work to compete with pathogenic bacteria for nutrition, and their growth is discouraged[iv].

The use of antibiotics in recent times has become indiscriminate, as they are used excessively. Overuse of antibiotics can lead to increased drug resistance, or even worse, altering the normal flora[v]. Fenugreek is an important medicinal plant, as studies have shown that fenugreek has an antibacterial and anti-inflammatory effect, and the antimicrobial properties of fenugreek seeds, both positive and negative for Gram stain, have been confirmed[vi]. Fenugreek seeds are toxic to bacteria and fungi, and the seeds are also used to treat stomach ulcers, intestinal inflammation, and urinary tract infections[vii]. In folk medicine in China, they are used to treat cancer.

The increase in keratitis with microorganisms associated with the increase in the use of contact lenses (medical and cosmetic) in recent years, as it is associated with the use of contact lenses many contaminated microorganisms, the most dangerous of them are Pseudomonas spp.., which are commonly attached to these lenses, as well as other genera such as: Bacillus spp., Corynebacterium, Staphylococcus, Klebsiella pneumonia, Nocardia spp., E.coli, and Haemophilus spp[viii]. Studies indicate that the number of normal conjunctival flora bacteria increased significantly after using the lenses for a month. The largest percentage of bacteria for most studies was coagulase-negative Staphylococcus bacteria isolated from the eyes of contact lens wearers[ix], studies also indicate that wearing contact lenses changes the microbial structure of the eye conjunctiva, making it more similar to that found in skin microbes, more research is needed to determine whether the structure of the microbiome provides less protection from infection in the eye[x].

Due to the lack of available studies in this regard, we decided to make it the title of the study, which aimed to:

- Isolation and identification of the natural flora present in the eyes.
- Confirm the diagnosis using the Al-faitek device and APPI 20E.
- Studying the effect of using contact lenses and eyeliner on the natural balance of the microbiome in the eyes.
- Testing the sensitivity of isolated species to antibiotics.
- Identifying the effectiveness of aqueous and alcoholic plant extracts of fenugreek plant for some pathogenic species and compared with antibiotics.

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ISSN: 2776-1010 Volume 4, Issue 1, Jan., 2023

Methods

A total of 160 samples were collected from 80 female students at Tikrit University, the number of samples that gave positive bacterial growth was 112, which constitutes 70% of the number of samples, while 48 samples from the total number of samples, i.e. 30%, did not show any bacterial growth, and this result is similar to what was stated by Bachrach (1953), where he obtained a positive bacterial growth for the natural flora of the eyes of healthy people, as the percentage reached 67.4% of the total samples, also, this result is similar to the results of 2003 (Bourcier), where the positive result of growth was 68%. This result also converged with what was obtained by Choudhury (2017), where the positive result of bacterial growth of the normal flora in healthy eyes was 69.4% of the total number of samples, and it also converged with what was found by Hsu 2014), where the positive percentage of bacterial culture of normal flora samples for healthy people was 77%, it is also very close to what was stated by Willcox (2013), where the positive growth of samples taken from the eyes of healthy people using contact lenses was 80%, and the percentage reached 82% according to (Grzybowski 2017).

Germinal culture of samples

Germinal culture of samples incubated with nutrient broth was carried out in the laboratory of the Department of Life Sciences, College of Education for Pure Sciences, according to the method of (Rajeshwari), three media were prepared for bacterial cultivation, they are, blood medium, Maconkey medium, and mannitol salt medium, then part of the sample incubated with the nutrient broth was transferred using a flame sterilized germ vector, with an amount full of the carrier loop to be planted on the dishes containing the culture media, then all the dishes were incubated in the incubator at 37 °C for 24 After that, the growing colonies were examined and the required isolation and diagnostic tests were performed.

Identification of Bacteria

Culture characteristics and Microscopically examination

The microscopic and cultural characteristics of the growing colonies on differential and nutritious culture media were studied, Bacterial isolates were diagnosed phenotypically based on the cultural and phenotypic characteristics, the size, shape, height, texture, smell, color and shape of the edges of the colonies were noted, as for the microscopic diagnosis, it was relied on a Gram stain to formulate the bacteria by taking a single colony from the bacterial culture and placing it on a glass slide containing a drop of distilled water, it was spread using a loop (lobe) in a spiral shape, then fixed by flame, then dyed with Gram stain according to the manufacturer's instructions, after which it was left for a few seconds to dry and was examined under a light microscope, information about them was confirmed, such as the shape of the bacteria, the way they were collected, their sizes, and whether they were negative or positive for dye [18].

Diagnosis of Gram- stain positive isolates was confirmed by the PHITEC device and the negative isolates were confirmed using the API 20E system. The following bacteria were found:

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Table (1): positive bacterial isolates and their numbers in the three groups

Gram-stain positive isolates	Contact lens set		Eyeliner and cosmetics set		Control group		Total percentage
Bacterial type	number	percentage	number	percentage	number	percentage	
S. warneri	11	13%	12	14%	1	1%	21.40%
S. Haemolyticus	11	13%	9	10.7%	_	_	17.80%
S.lentus	1	1.1%	6	7.1%	1	1.1%	7.10%
S. hominis	2	2.3%	3	3.5%	8	9.5%	11.60%
S. vitulinus	_	_	3	3.5%	4	4.7%	6.20%
S. aureus	2	2.3%	_	_	_	_	1.70%
K. kristinae		_	_	_	6	7.1%	5.30%
A. viridans	_	_	4	4.7%	_	_	3.50%

Table (2): The numbers and percentages of negative bacterial isolates in the three groups

Gram-stain negative isolates	Contact lens set		Eyeliner and cosmetics set		Control group		Total percentage
Bacterial type	number	percentage	number	percentage	number	percentage	
E. coli	4	12%	2	%6	_	_	5.3%
Klebsiella	4	12%	_	_	1	3%	4.4%
oryzihabitans.P	5	15%	4	12%	_	_	8%
Myroides	5	15%	_	_	_	_	4.4%
Berkshire	_	_	1	3%	_	_	0.8%
E. americana	_	_	1	3%	_	_	0.8%
E. sakazakii	_	_	1	3%	_	_	0.8%

Table (3): Biochemical tests on Gram-stain negative bacteria

Gram-stain negative bacteria	Indol	Citrate	Oxidase	Catalase	Urease	Methyl red	H_2S	Motility	Vogesproskour
E. coli	+	_	_	+	V	+	_	+	_
Klebsiella	-	+	_	+	+	_	-	_	+
oryzihabitans.P	_	-	_	+	_	_	_	+	_
Myroides	_	+	+	+	+	_	+	_	+
Berkshire	-	+	+	+	+	-	-	+	-
E. americana	-	+	-	+	-	+	_	-	+
E. sakazakii	-	+	-	+	-	-	-	+	+

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Table (4): Biochemical tests for Gram-stain positive bacteria

Bacteria	Catalase	Oxidase	Coagulase	Hemolysine	Urease	Novobiocin	Motility
S. aureus	+	_	+	+	+	-	-
S. vitulinus	+	+	_	+	-	+	
S.Haemolyticus.	+	_	_	+	_	-	-
S. hominis	+	_	_	_	-	+	
S. warneri	+	-	-	V	+	-	
S.lentus	+	-	_	-	_	+	
A. viridans	-	-	-	+	-	-	-
K. kristinae	+	-	-	-	+	+	-

Table (5): The ability of bacterial species to ferment some types of sugars

Bacterial isolates	Maltose	Sucrose	Lactose	Glucose	Mannitol	Mannose
E. coli	-	V	+	+	+	_
Klebsiella	+	+	+	+	+	+
oryzihabitans.P	+	+	-	+	+	-
Myroides	-	-	-	-	-	-
Berkshire	+	-	+	+	-	-
E. Americana	+	-	+	+	+	+
E. sakazakii	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+
S. vitulinus	+	+	-	+	+	-
S. Haemolyticus.	+	+	V	+	v	-
S. hominis	+	+	-	+	-	-
S. warneri	+	+	V	_	V	-
S. lentus	-	+	+	+	-	+
A. viridans	+	+	+	_	+	+

Plant Extracts

Fenugreek plant Trigonella foenum-graecum was collected from Attarin in the city of Tikrit during the month of October, the plant samples used in the study were cleaned, then, the fenugreek seeds were ground with an electric mill and kept in sterile and sealed containers in moisture-free conditions until the plant extracts started to work.

Preparation of aqueous extract of fenugreek plant

The aqueous extracts of the plants used in the research were prepared by dissolving 40 g of the plant form powder (fenugreek seeds) in 160 ml of distilled water, i.e. (4:1) w/v, then the sample is placed in a blender, then the mixture is placed in the refrigerator for at least an hour for soaking purposes, then it is placed in a vibrating incubator at a temperature of 37 degrees for 24 hours, then it is filtered through several layers of sterile gauze and then filtered again through a Buechner funnel using filter paper

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(Whatman no1) with vacuuming by a vacuum device (Vacum) to get rid of the non-pulverized parts and fiber residue, thus, the crude aqueous extract was prepared, and then the extract was dried by cooling under a sieve pressure using a lypholizer, after drying, the samples were placed in tightly closed and sterilized glass bottles and kept in moisture-free conditions by freezing until use[19].

Preparation of alcoholic extracts of fenugreek

Dry fenugreek seeds were obtained from two apothecaries in the city of Tikrit, then crushed using a household electric grinding device, the aqueous and alcoholic Extract Crude was prepared according to the method mentioned in Al-Khafaji, where 100 g of vegetable powder was weighed and soaked in 500 milliliters of ethanol at a concentration of 70% to obtain the ethanolic extract, the mixture was left in the incubator shaker at 37°C for 24 hours, then the wort was filtered using Whatman No1 filter paper and the solution was evaporated by the Vaccum Evaporator Rotary to obtain a concentrated solution under the influence of sieve pressure, then the solution was dried in an electric oven at a temperature of 40 °C in the presence of a circulating air current until a dry powder was obtained, then the resulting powder was kept in a sterile glass vial until use in dry and cool conditions[20] .

Sterilization of the aqueous extract of the fenugreek plant

The aqueous extract was prepared by weighing 1 gm of the dry extract and dissolving it in 10 ml of sterilized distilled water to produce an aqueous extract with a concentration of 100 mg / ml, and calculating the standard concentration for the rest of the dilutions required for the study, a solution with a concentration of 75%, 50%, and 25% was obtained by dissolving 0.75 g, 0.50 g, and 0.25 g, respectively, of the dry extract powder in 10 ml of distilled and sterilized water, the fenugreek extract was also filtered by layers of medical gauze, and then filtered by filter paper, because due to the high viscosity of the fenugreek solution, fine membrane filters cannot be used, as it does not pass through them even when using large sizes of them.

Sterilization of the alcoholic extract

The alcoholic extract was prepared by dissolving 1 gm of the dry alcoholic extract in 10 ml of distilled water to obtain a concentration of 100%, then the mixture was sterilized by pasteurization at a temperature of 62.8 for 30 minutes. [21] Thus, the standard concentration was obtained, which was used to obtain the rest of the dilutions.

Testing the inhibitory effectiveness of plant extracts of fenugreek plants against some bacterial species identified in the study that are resistant to antibiotics.

The agar diffusion method was used using the wells method to test the sensitivity of the isolated bacteria to different concentrations of the previously prepared plant extract, the method was done after spreading (0.1) ml of the bacterial suspension on the medium with soap, then, five holes with a diameter of 5 mm were made using a Cork bore, with equal dimensions, in the center of the solid Mueller Hinton, 4 holes were made for the extracts and a hole in the medium containing distilled water was filled with plant solutions at an amount of (0.2) or (0.1) ml for each hole, according to the thickness of the medium,

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then the dishes were left in the refrigerator for one hour to ensure the spread of the plant solutions, then the dishes were incubated at (37) C° for a period of (24) hours and the results were read by measuring the diameter of the inhibition zone in mm.

Effect of plant extracts on bacterial isolates

Our results revealed the importance of plant extracts to inhibit resistant bacteria, which have become a threat to human health, and thus reduce the potential toxic effects of antibiotics on the human body, plant extracts have great potential as antimicrobial compounds against microorganisms, thus, they can be used in the treatment of infectious diseases caused by resistant microbes. Plants and botanical products have been used extensively throughout history to treat medical problems, studies have been conducted to extract various natural extracts to examine antimicrobial activity 22, plant extracts may act to inhibit bacteria by a different mechanism than that of antibiotics used and may have antibacterial therapeutic value against multidrug-resistant bacterial strains, the effect of the aqueous and alcoholic extract of fenugreek on bacterial isolates was studied and it was noted that the inhibitory activity of the extracts varied according to the extraction solvent and test microorganism[10].

Inhibition of aqueous extracts

The results were recorded and compared with other studies, with regard to the aqueous extract of fenugreek, concentrations of 100%, 75%, 50% and 25% of the extract were used, the results of our study showed the effect of the aqueous extract of fenugreek seeds at a concentration of 25%, its inhibition diameters on bacterial species ranged between 8 and 11 mm, with the highest inhibition diameter reaching S. hominis, where the diameter of inhibition was 11 mm, and the least diameter of inhibition was 8 mm for S. warneri bacteria, as for the rest of the bacterial species, the diameters of inhibition were between 9 and 10 mm. As for the diameter of 9, it was for p.oryzihihabitans, S. aureus, E. sakazakii and S.lentus, as for the diameter of 10 mm, it was for E. coli, Klebsiella, Myroides, Berkshire, E. Americana, and A. viridans, K. kristinae, S. vitulinus, and S. haemolyticus.

These results did not agree with Souad (2015), as the bacterial isolates showed resistance to the effectiveness of the cold aqueous fenugreek extract at a concentration of 25%, and for the second concentration, which is a concentration of 50%, its inhibition diameters ranged between 10 and 13 mm, where the least inhibition diameter was when the bacterium p.oryzihihabitans reached 10 mm, the highest inhibition diameter, which was 13 mm, was found in A. viridans, S.warneri and E.coli, as for the rest of the genera, most of them were 12 ml, namely Klebsiella, Myroides, Berkshire, E. Americana, S. aureus, S. vitulinus, S. hominis, and S. S. aureus, S. lentus, K. kristinae, S. vitulinus, and S. haemolyticu, as for diameter 11, it was for E. sakazakii bacteria. For the third concentration, which is a concentration of 75%, the inhibition diameters for this concentration ranged between 13_18, the least inhibition diameter was 13 mm for E. Sakazakii and P.oryzihihabitans, as for the highest inhibition diameter, it was for S.warneri bacteria, Berkshire bacteria, and E. Americana, which reached 18 mm, as for diameter 16, it was found in Klebsiella and S.haemolyticus bacteria, as for the rest of the bacterial genera, they

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were as follows, Myroides, A. viridans, and S. aureus, K. kristinae and E.coli inhibition diameter is 15 mm, as for diameter 14, it was in S.lentus, S. hominis, and S. vitulinus.
With regard to the highest concentration, which is 100%, its inhibition diameters ranged from 18 to 20 mm, with the lowest diameter being 18 mm for Klebsiella and P.oryzihabitans. hominis. s, S. aureus, and E. sakazakii, while the highest diameter of inhibition was found in each of E. coli, Berkshire, E. Americana, S.warneri, S.lentus, K. kristinae, and S. Vitulinus, S.haemolyticus and A. viridans for Myroides, the diameter was 19 mm.

Effect of alcoholic extract on bacterial isolates

Regarding the alcoholic extract of fenugreek, its inhibition drops were recorded as follows:
As for the lowest concentration of fenugreek, which is 25%, this concentration did not act on bacteria at all, and no inhibitory diameter was recorded, as for the second concentration of 50%, its inhibition was weak, as the diameters ranged from 10 mm to 15 mm only, where the diameter was the least, which is 10 mm in E.coli, P. oryzihabitans, S.vitulinus, A. viridans, and S. hominis, as for the diameter of 15 mm, it was for Berkshire bacteria. As for the rest of the bacterial genera, it ranged between these two concentrations, and it did not inhibit S. aureus and S. warnery, with regard to the third concentration, 75%, the concentrations ranged between 12 and 18 mm, and the diameter was the smallest for E.coli bacteria, as for the largest diameter of 18 mm, it was for each of Klebsiella, Berkshire, E. Americana, and the rest of the species, it ranged between the two concentrations and in different numbers.

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