

Study of 16S rRNA gene expression of bacteria causing conjunctivitis

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Conjunctivitis, *Staph-epidermidis*, *E. coli*, black tea, vancomycin.

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ABSTRACT

One hundred samples of patients with conjunctivitis who visited the Fallujah Teaching Hospital's eye consultation were included in the study. For both genders, the age range covered was 9 months to 77 years. The study found that 87% of the samples were positive for bacterial growth, while 13% were negative for it. 56.32% of them were for males and 43.67% for females. A variety of bacteria were isolated, including *Staphylococcus aureus*, *Staph-epidermidis*, and *E. coli*. The study demonstrates that the minimum inhibitory concentration (MIC) of each black tea aqueous extract and vancomycin was 12.5, which gives 100% inhibition. The inhibition effects of synergistic black tea extract and vancomycin were at concentrations of 6.2 giving 100% inhibition. Also, black tea extract has a beneficial effect on the gene expression of the 16S rRNA gene of *E. coli* bacteria in cooperation with untreated samples, which is down-regulation. Furthermore, the black tea extract affected all types of *Staph-epidermidis* bacteria by increasing their gene expression (upregulation) in cooperation with the untreated sample.

Introduction

A considerable portion of the global population suffers from common eye disorders. Even though some of these issues may initially seem minor, if these conditions are not treated, it can lead to major eye issues and irreversible visual damage. Due to their ease of transmission from one person to another and from the patient's infected eye to the healthy eye, both bacterial and viral conjunctivitis are regarded as highly infectious [1]. In medicine, one of the most prevalent eye infection is bacterial conjunctivitis. The majority of cases are acute, personal to the patient, and do not contribute significantly to the illness. However, because of its serious side effects, such as missing work or school, it has a big impact on society. In this situation, using antibiotics could help the patient resume regular activities and lessen the consequences of the illness [2].

When bacterial conjunctivitis is accompanied by purulent or mucous discharge, it is considered chronic[3]. Bacterial conjunctivitis typically lasts seven to ten days. Therapeutic intervention becomes important in patients with immunodeficiency or contact lens wearers. Since bacterial conjunctivitis self-heals after being treated for 7–14 days, using antibiotics to treat it is thought to be effective in at least 60% of cases, including the acute variety. [4]. However, the excessive use of antibiotics has led to the emergence of new antibiotic-resistant bacterial strains, which the World Health Organization has classified as a serious threat to the future [5]. during a period when the efficacy of artificial antibiotics is also declining. The development of novel natural compounds that can be employed as antibiotic substitutes and are distinguished by their exceptional efficacy and chemical variety represents the best chance for the treatment of bacterial illnesses [6].

Materials and Methods

Sample collection

At the Fallujah Teaching Hospital consultation, 100 swabs were collected from the conjunctiva of the

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patient's eyes. The swabs included male and female ages ranging from 9 months to 77 years. Using sterile cotton swabs, samples were taken after a clinical evaluation by a professional doctor. Following their transfer, the samples were cultivated on a carrier medium (brain heart broth) in the microbiology lab at Fallujah Hospital. Then, underwent incubation for 24 hours at 37°C. The samples were subsequently grown on Maconkey agar and blood agar media. It was incubated aerobically for 24 hours at a temperature of 37°C [7].

Microscopic examination

Gram stain was applied to swabs obtained from bacterial colonies, which were subsequently examined under an optical microscope [8]. Biochemical tests: the catalase test, oxidase test, blood coagulation test, growth test on menthol salt, indole production, citrate test, and methyl red MR test, were performed, and the results were interpreted by the information provided in [9].

Sample treatment

Hot aqueous extract of black tea: 20 grams of black tea leaves were dissolved in 1000 ml of sterile, distilled water to produce the aqueous extract. Following that, the mixture was combined on a plate at 60°C using a magnetic stirrer. After that, filter paper was used to filter the solution. To dry it out, it was put in an oven set to 40°C. Testing the effect of black tea extract on bacteria isolated from patients.

Resistance to Antibiotics

Antibiotic susceptibility testing using the MIC method [10]. To assess the extract's synergistic relationship with the antibiotics utilized in the study, the minimum inhibitory concentration (MIC) is determined. A range of values, from 0.7 to 400 mg/ml, was utilized to calculate the minimal inhibitory concentration.

PCR amplification of 16S rRNA gene

Quantitative analysis of gene expression. Quantitative real-time PCR (qRT-PCR) was used to evaluate gene expression, with the *16S rRNA* housekeeping gene serving as an internal control to standardize the expression levels across samples. The organization (TRANS) used the Perfect star Green qPCR super mix

kit for the experiment. The cDNA samples were produced and put in a tube holder. The reaction mixture was then created based on the additions specified in Table (1), and the primer information indicated in Table (2) was used. In order to assess the variability in gene expression at the RNA level across the various samples, the Ct value of each sample was compared to that of the positive control group using the " $\Delta\Delta C_t$ " technique as described by Yuan et al. (2006)[11].

Table 1: Protocol RT_qPCR tube

Primer	Sequence	Primer sequence5' - 3'	Tm (°C)	GC%
<i>Staphylococcus epidermidis</i>	F	ATCCTTGCC ACGTTCTGTTA		
	R	TGGCACGCT GACACCATTAT		
<i>E coli</i>	F	AGCAGCTGC GCTTATTCTCT		
	R	GGAACAACC ACGACATTGGC		
<i>16s RNA Reference gene</i>	F	CAGCTCGTG TCGTGAGATGT	62	55.0
	R	G CGTAAGGGC CATGATGACTT	60	50

Table 2: The Primers that used for RT_qPCR

Reagent	Volume 20 µl
GoTaq® qP CR Master Mix (2X)	10 µl
Forward Primer (20X)	0.5 µl
Reverse Primer (20X)	0.5 µl
Supplemental CXR Reference Dye	0.2µl per reaction
cDNA	5 µl
Nuclease-Free Water	to a final volume of 20µl

3. Result and Discussion

Following the initial examination, 87 samples, or 87% of the total, tested positive for bacterial growth. Thirteen of the samples were negative for bacterial growth. The findings indicated that 56.32% of males had an infection. Figure (1) indicates that the infection rate in females was 43.67%. However, the study also revealed

that the age range of 1 to 15 years old had the highest infection rate, with a rate of 33.33%. The age group (15–30) years old came next, with a percentage of (28.73%). *Staphylococcus aureus* was found to be the predominant strain in the investigation, accounting for 59 isolates and a proportion of 67.81. The percentages and total number of bacterial isolates are displayed in Table (3).

Table (3) Isolated species and their percentages

No.	Isolated species	number	percentage %
1	<i>Staphylococcus aureus</i>	59	67.81
2	<i>Staphylococcus epidermidis</i>	10	11.49
3	<i>E coli</i>	15	17.24
4	<i>Staphylococcus lentus</i>	7	8.04
5	<i>Staphylococcus haemolyticus</i>	4	4.59
6	<i>Pseudomonas auroginosa</i>	3	3.44

The results of this study supported the findings of Hameed (2020)[12], who found that patients with conjunctivitis had a 77.6% infection rate with

Staphylococcus aureus bacteria. [13] also demonstrated that the usage of contact lenses and lens implantation for patients with cataracts were the primary causes of most patients' bacterial infections, particularly those caused by *Staph. aureus* and *Staph. epidermidis* bacteria. This resulted from these bacterial species' capacity to generate biofilms. The infection rate in males and females is displayed in Figure (1).

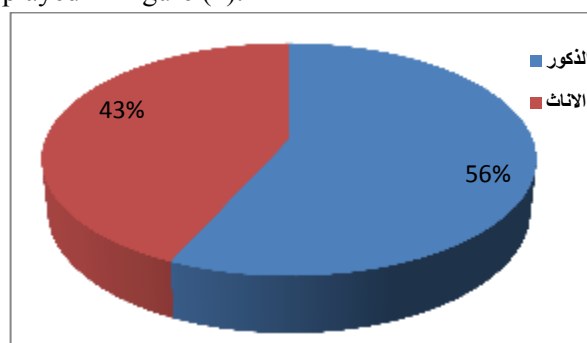


Figure 1: Shows the infection rate in males and females

Table 4: Concentrations of black tea extract and their effect on the bacteria under study

No		Concentrations of extract									
		400	200	100	50	25	12.5	6.2	3.1	1.5	0.7
1	<i>Staph-epidermidis</i>	-	-	-	-	-	-	+	+	+	+
2	<i>Staph-epidermidis</i>	-	-	-	-	-	-	+	+	+	+
3	<i>Staph-epidermidis</i>	-	-	-	-	-	-	+	+	+	+
4	<i>Staph-epidermidis</i>	-	-	-	-	-	-	+	+	+	+
5	<i>E.coli</i>	-	-	-	-	-	-	+	+	+	+
6	<i>E.coli</i>	-	-	-	-	-	-	+	+	+	+
7	<i>E.coli</i>	-	-	-	-	-	-	+	+	+	+
8	<i>E.coli</i>	-	-	-	-	-	-	+	+	+	+

The sign (+) indicates the presence of bacterial growth, and the sign (-) indicates the absence of bacterial growth.

The impact of a heated black tea aqueous Extract on the studied bacteria

Table (4) demonstrates the antibacterial properties of black tea extract. On the tested bacterial species, the minimum inhibitory concentration (MIC) of 12.5 demonstrated 100% inhibition. This suggests that black tea inhibits the growth of bacteria, which is in line with the findings of both studies [14, 15]. This is consistent with research [16] that showed the polyphenols in tea could either kill or stop the growth of bacteria that cause

disease, including *staphylococcus* and *salmonell*. The presence of bacterial growth is denoted by the sign (+), and the absence of bacterial growth is indicated by the sign (-).

Vancomycin's impact on the bacterial species under study

This antibiotic showed a significant effect on the bacterial species studied, as the 12.5 concentration was 100% inhibitory. While the 6.2 concentration was 50% inhibitory. According to Table (5), concentration 3.1 had

an inhibiting effect of 12.5%. This is in line with a finding made by Stogios and Savchenko in 2020, which showed that the antibiotic vancomycin clearly inhibits the growth of bacteria.

Table 5: Shows the effect of vancomycin on the bacterial isolates under study

No		Concentration of vancomycin									
		400	200	100	50	25	12.5	6.2	3.1	1.5	0.7
1	<i>Staph-epidermidis</i>	-	-	-	-	-	-	-	-	+	+
2	<i>Staph-epidermidis</i>	-	-	-	-	-	-	+	+	+	+
3	<i>Staph-epidermidis</i>	-	-	-	-	-	-	+	+	+	+
4	<i>Staph-epidermidis</i>	-	-	-	-	-	-	+	+	+	+
5	<i>E.coli</i>	-	-	-	-	-	-	-	+	+	+
6	<i>E.coli</i>	-	-	-	-	-	-	+	+	+	+
7	<i>E.coli</i>	-	-	-	-	-	-	-	+	+	+
8	<i>E.coli</i>	-	-	-	-	-	-	-	+	+	+

The sign (+) indicates the presence of bacterial growth, and the sign (-) indicates the absence of bacterial growth.

Black tea extract and vancomycin's synergistic impact on the examined bacterial species

The findings demonstrate that the addition of black tea extract to the antibiotic enhanced its inhibitory efficacy against the investigated bacterial species, vancomycin. An inhibition rate of 87.5% was observed at the minimum inhibitory concentration (MIC) of 3.1. According to Table (1-6), the inhibition rate at concentration 6.2 was 100%, and at concentration 1.5 it was 12.5%. The type of chemicals, alkaloids, titins, flavins, and other substances with antibacterial activity that are present in the plant is what gives it its inhibitory efficiency [17].

The effect of black tea extract on the gene expression of *Ecoil* and *Staph-epidermidis* bacteria:

Using the minimum inhibitory concentration (MIC) approach, RNA was isolated from the bacteria following their treatment with black tea extract. The RNA was then transformed into cDNA, which allowed the quantitative polymerase chain reaction to be finished. Black tea extract has a beneficial effect on *E. coli* bacteria, as demonstrated by Tables 1–7. All *E. coli* bacterium types under

investigation had lower levels of gene expression after treated with tea extract from untreated.

Table 6: Synergistic relationship of tea extract and the antibiotic vancomycin

		Concentration of tea extract and vancomycin(1-6)									
N	o	400	200	100	50	25	12.5	6.2	3.1	1.5	0.7
1	<i>Staph-epiderma dis</i>	-	-	-	-	-	-	-	+	+	+
2	<i>Staph-epiderma dis</i>	-	-	-	-	-	-	-	-	+	+
3	<i>Staph-epiderma dis</i>	-	-	-	-	-	-	-	-	+	+
4	<i>Staph-epiderma dis</i>	-	-	-	-	-	-	-	-	+	+
5	<i>E.coli</i>	-	-	-	-	-	-	-	-	-	+
6	<i>E.coli</i>	-	-	-	-	-	-	-	-	+	+
7	<i>E.coli</i>	-	-	-	-	-	-	-	-	+	+
8	<i>E.coli</i>	-	-	-	-	-	-	-	-	+	+

Table 7: Displays how black tea extract affects the bacteria *E. coli*.

		<i>E. coli</i>	16s	Δct	folding
1	before	15.65	16.34	-0.69	1
2		18.2	15.65	2.55	1
3		17.59	15.51	2.08	1
4		17.04	16.54	0.5	1
5	after	17.44	16.31	1.13	0.283220971
6		23.44	16.09	7.35	0.035896824
7		24.92	16.69	8.23	0.014082038
8		25.01	16.02	8.99	0.002781348

Table (1-8) illustrates how the black tea extract affected all types of *Staph-epidermidis* bacteria by increasing their gene expression (upregulation) in comperation with untreated sample.

Table 8: Effect of black tea extract on *Staph-epidermidis* bacteria

No.		<i>S. ep</i>	16s	Δct	folding
1	before	21.04	17.52	3.52	1

2	26.94	17.37	9.57	1
3	18.26	17.13	1.13	1
4	18.56	19.37	-0.81	1
5	after 16.55	17.43	-0.88	21.11212657
6	16.75	17.55	-0.8	132.9084829
7	15.06	17.69	-2.63	13.547925
8	15.08	22.93	-7.85	131.5985698

The study done by (Turkmen *et al.*,2007)[18] show black tea contain markedly higher amounts of total polyphenol and antioxidant activity and antibacterial effect. Black tea contain polyphenol compounds, the most frequent are catechins.

The most important of the catechins are epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) [19].

The study done by Yang *et al.*,(2018)[20], show effect of epigallocatechin gallate on gene expression of *E.coli*

The bactericidal action of EGCG was a result of increased intracellular reactive oxygen species and blunted adaptive oxidative stress response in *E. coli* due to the co-administration of antioxidant N-acetylcysteine, and not on account of exogenous catalase [21] [22] evaluated the microbiologic effects of black tea, compared to green tea, alone and in conjunction with selected antibiotics against *E. coli*. At a concentration of 25 mg/mL, both black tea and green tea completely inhibited *E. coli* growth after 5 and 7 h. Both tea extracts had either synergistic or antagonistic effects at different concentrations on selected antibiotics.

Conclusions

The study concluded that Bacterial infections are one of the causes of conjunctivitis, which effect male rather than female. The synergistic effect of vancomycin and black tea more inhibit bacterial growth than each compound alone. On the other hand black tea extraction down regulated 16s rRNA and up regulated of 16s rRNA of *Staph-epidermidis*

Conflicts of interest

There are no conflicts of interest.

Funding source

The authors did not receive any source of fund.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

Author Declaration

Institutional Review Board Statement:

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Department of Biology/ College of Education for Girls/University of Anbar, No. 20468 on 22 / 8 /2022.

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دراسة التعبير الجيني لجين *16S rRna* للبكتريا المسببة التهاب ملتحمة العين

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الخلاصة

تضمنت الدراسة 100 عينة من المرضى المراجعين لاستشارية العيون في مستشفى الفلوجة التعليمي من المصابين بالتهاب ملتحمة العين Conjunctivitis للأعمار بين (9 اشهر – 77 سنة) للذكور والإناث بينت نتائج الزرع البكتيري أن 87 % من العينات كانت موجبة للنمو البكتيري أما العينات 13 % فكانت سالبة للنمو البكتيري ، تم عزل انواع مختلفة من البكتريا منها *E. coli* ، *Staph-epidermidis* وبكتريا *Staphylococcus aureus* أظهرت الدراسة ان الحد الأدنى من التركيز المثبط الأدنى MIC لكل من المستخلص المائي للشاي الاسود ومضاد vancomycin كان 12.5 مما يعطي تثبيط بنسبة 100% ، كان التركيز المثبط لتأثير العملية التآزرية بين مستخلص الشاي الاسود ومضاد vancomycin 6.2 مثبط بنسبة 100% ، كما تبين ان لمستخلص الشاي الاسود تأثير فعال على عملية التعبير الجيني لجين *16S Rna* الخاص ببكتريا *E. coli* مقارنة مع العينات الغير معالجة بالمستخلص . كما اثر مستخلص الشاي الاسود على جميع انواع بكتريا *Staph-epidermidis* عن طريق زيادة التعبير الجيني مقارنة بالعينات الغير معرضة لتأثير المستخلص .

الكلمات المفتاحية : الملتحمة ، الاشريكية القولونية ، الشاي الاسود ، الفانكوميسين ، المكورات العنقودية الجلدية