Antimicrobial Effects of Black Tea (*Camellia sinensis*) on *Pseudomonas Aeruginosa* Isolated from Eye Infection

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Abstract

This study was conducted to evaluate the effects of black tea on *Pseudomonas aeruginosa* isolated from eye infection. One hundred samples (corneal scrapings) were obtained. Approximately, 77% of the cases were due to contact lens wear followed by 15 % trauma and 8% with unknown history. The isolates identified as *P. aeruginosa* were 30% (23/77 CL) and 25% (2/8 Unknown). On the other hand, the Kirby-Bauer antibiotic sensitivity assay showed that 100% of the isolates were sensitive to Neomycin, Gentamicin and Amikacin. While 91.6% were sensitive to Carbenicillin and Ceftriaxone; 66.6% were sensitive to Cefotaxime and 0% were sensitive to Tertacycline. Only two isolates were found to be multidrug resistant. Screening for some Pseudomonas virulence factors such as hemolysin and protease showed that all the isolates had the ability to produce Beta hemolysin and digested casein due to protease secretion. For adhesion ability using Christensen's method, 8.33% were recorded as strong (+++), 41.66% were moderate (++) while 50% were weak (+).In contrast black tea (*Camellia sinensis*) was examined for its antimicrobial activity. The agar-well diffusion method was used for the concentrations 100, 200, 300, and 400mg/ml respectively. Results showed that the minimum inhibitory concentration of tea alcohol extract was 400mg/ml with inhibition zone of 20mm. The extract decreased the bacterial viable count since it showed a visible decrease to $<5\times10^6$ Colony Forming Unite (CFU)/ml after 24 hours of incubation. Black tea extract also had the ability to completely inhibit *Pseudomonas* growth on blood agar and inhibited protease activity and adhesion. There were also differences in Congo red binding seen in bacterial cell suspensions cultured in growth media that contained tea extract. The synergistic activity of tea extract with antibiotics has changed the resistance of *P. aeruginosa* (without the tea) to sensitive (in presence of tea extract).

Keywords: Keratitis, *Pseudomonas aeruginosa*, Contact lenses, Antibiotic Sensitivity test, Virulence factors, Adhesion, *Camellia sinensis*, Agar-well diffusion method, Synergistic Activity.

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الفعالية الضد ميكروبية للشاي الأسود على بكتريا *Pseudomonas aeruginosa* المعزولة من أصابات العين

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الخلاصة

اجريت هذه الدراسة لتقييم تأثير الشاي الاسود على بكتريا *Pseudomonas aeruginosa* المعزولة من خمج العين. تم الحصول على مائة عينة (كشطات القرنية). وجد تقريبا إن 77% من تلك الحالات تعود إلى ارتداء العدسات اللاصقة تلتها 15% من حالات الجرح أو الصدمة و 8% كانت لأسباب غير معروفة. وان العزلات المشخصة *Pseudomonas aeruginosa* كانت 30% (23/ 77عدسات لاصقة) و 25% (2 / 8 غير معروفة).من الناحية الأخرى , اظهر فحص Kirby-Bauer للحساسية تجاه المضادات الحياتية بأن 100% من العزلات كانت حساسة للنيوميسين, جينتاميسين, واميكاسين. في حين ان 91.6% كانت حساسة للسبروفلوكساسين والسفترايكسون و66.6% كانت حساسة للسيفوتاكسيم و 0% حساسة للتتراسايكلين. وجد ان عزلتان فقط متعددة المقاومة للمضادات. اظهر التحري عن بعض عوامل الفوعة ل Pseudomonas مثل الهيمولايسين والبروتييز بأن جميع العزلات كان لها القابلية على إنتاج الهيمولايسين نوع بيتا و قابليتها على هضم الكازئيين نتيجة لإفراز البروتييز. وباستخدام طريقة Christensen للقابلية على الالتصاق, سجلت 8.33% قوية (+++) , 41.66% متوسطة القوة (++) في حين ان 50% كانت ضعيفة (+).فضلا عن ذلك تم اختبار الفعالية الضدمايكروبية للشاي الاسود (*Camellia sinensis*). استعملت طريقة الانتشار بالحفر (The agar-well diffusion method) للتراكيز (100, 200, 300 و 400 ملغم /مل) على التوالي. وأظهرت النتائج بأن التركيز المثبط الأدنى (MIC) للمستخلص الكحولي للشاي الاسود كان 400 ملغم /مل حيث بلغ قطر منطقة التثبيط 20ملم. وكان هنالك تأثير واضح للمستخلص على حيوية البكتريا حيث ظهر إن الشاي الاسود قد خفض العد الحيوي البكتيري إلى اقل من 000 000 5 وحدة مكونة للمستعمرة بعد مرور أربع وعشرون ساعة على حضن المزروع البكتيري. المستخلص الكحولي للشاي الاسود أيضا كان له القدرة على التثبيط الكلي لنمو *Pseudomonas* على وسط الدم وفعالية البروتييز وعلى الالتصاق. لوحظ وجود اختلافات في ربط احمر الكونغو (Congo red) في العوالق البكتيرية المزروعة على أوساط حاوية على مستخلص الشاي. الفعالية التأزرية للشاي الاسود مع المضادات الحياتية غيرت مقاومة بكتريا *Pseudomonas aeruginosa* (بغياب الشاي) إلى حساسة (بوجود الشاي).

Introduction

Bacterial keratitis is an infection and inflammation of the cornea that cause pain, reduced vision, light sensitivity and tearing or discharge from the eye that can, in severe cases cause loss of vision. Bacterial keratitis progresses rapidly and corneal destruction may be complete in 24 - 48 hours with some of the more virulent bacteria. It may involve the center of the cornea or the peripheral part of the cornea (that portion closest to the sclera) or both. Keratitis may affect one eye or both eyes. Keratitis may be mild, moderate, or severe and may be associated with inflammation of other parts of the eye [1].Bacterial keratitis accounts for approximately 65% to 90% of all corneal infections [2].

Until recently, most cases of bacterial keratitis were associated with ocular trauma or ocular surface diseases. However, the widespread use of contact lenses has dramatically increased the incidence of contact lens (CL) related keratitis [3]. The spectrum of bacterial keratitis can also be influenced by geographic and climatic factors. Many differences in keratitis profile have been noted between populations living in rural or in city areas, in western, or in developing countries [4]. Ulcerations of the cornea may occur, a condition known as ulcerative keratitis. Many patients have a poor clinical outcome if aggressive and appropriate therapy is not promptly initiated [5]. *In vitro* studies of antibacterial susceptibility tests by various authors have shown that

fluoroquinolones were used as an effective monotherapy for many patients with microbial keratitis as they provide a good coverage for most of the gram positive and gram negative bacteria [6].However, reports on emergence of resistance to fluoroquinolones have recently been published with special emphasis to *Pseudomonas* spp. [7]. This resistance has increased due to in discriminated use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists to search for new anti-microbial and anti-inflammatory substances from various sources, such as medicinal plants [8].

The tea plant *Camellia sinensis* is native to Southeast Asia but is currently cultivated in >30 countries around the world. *Camellia sinensis* is the species of plant whose leaves and leaf buds are used to produce tea [9]. It is of the genus *Camellia*, a genus of flowering plants in the family Theaceae. Tea is consumed worldwide, although in greatly different amounts.

The tea is an infusion of leaves that has been consumed for centuries as a beverage and is valued for its medicinal properties. The phytochemical screening of tea revealed the presence of alkaloids, saponins, tannins, catechin and polyphenols [10]. In old China, tea was first used as an antidote and in India as a kind of medicine for treatment of diarrheal disease [11]. In recent years there are some reports, mostly from Japan, dealing with the antibacterial activity of tea. It is well known that tea polyphenols are responsible for the antibacterial activities of various tea products. Polyphenolic compounds make up some 30% of the dry weight of flush and black tea leaves. The simplest compounds are catechins which are well characterized isoflavanoids, mainly consisting of four compounds, (-)-epicatechin (EC), (-)-epigallocatechin (EGC), -epicatechin gallate ECG, and -epigallocatechin gallate (EGCG). The larger molecules in the class include theaflavins and thearubigins, which are oxidation and polymerization products of simple isoflavanoids and main polyphenolic compounds in black tea [12]. In traditional Chinese and Indian medicine systems, tea has been used as a stimulant, diuretic, astringent, and to improve heart health. Other traditional uses of tea include treating flatulence (gas), regulating body temperature and blood sugar, promoting digestion, and improving mental processes. In Japanese folkloric medicine tea is used to prevent tooth decay. Tea has been considered to be anti-inflammatory, antioxidative and anti carcinogenic [13].

Our study spotlights the effects of black tea extract alone as well as with antibiotics against *Pseudomonas aeroginosa* isolated from eye infection (keratitis).

Materials and Methods

Isolation and Identification

One hundred corneal scrapings were obtained by an ophthalmologist using a sterile bent-tipped needle from patients with suspected microbial keratitis presenting to IBN Al-Haytham Teaching Eye Hospital in Baghdad, Iraq. There were only twenty-five positive cultures.

The material obtained from the scrapings was inoculated onto sheep blood agar, MacConkey agar, Brain heart infusion broth [14]. In a positive culture, the samples were further sub-cultured onto Cetramide agar to obtain pure colonies which were then tested with routine microscopically and biochemical tests for identification and finally confirmed by the API 20E profile index and PIBWin computer database [15 and 16].

Antibiotic Sensitivity test

In *vitro* susceptibility testing was done by Kirby-Bauer disk diffusion method for the positive isolates. Amikacin (10mcg), Carbenicillin (100mcg), Cefotaxime (10mcg), Ceftriaxone (10mcg), Gentamicin (30mcg), Neomycin (30mcg), Tetracycline (30mcg) discs were used. They were purchased from Bioanalyse and were consistently tested for efficacy against the isolates. The results were interpreted according the refrences[17 and 18].

Detection of Some bacterial virulence factors

1- Hemolysin production was detected on blood agar plates that were inoculated with the test organism and then incubated at 37°C for 24hours. The plates were then examined for the

presence of alpha or beta haemolysis around the colony [19].
2- The skimmed milk agar was used to screen the protease activity [20].

Adhesion Ability Detection

It was detected by two methods:
1- Congo red agar: Congo red agar previously prepared was inoculated and incubated at 37°C for 24 h. A positive result was indicated by black colonies on the surface. Non-slime producing strains developed red colonies. Each plate was interpreted by two different observers
2- The Tube adherence method (Christensen's method) a loop of isolates from a blood agar plate was inoculated into a glass tube containing 5 ml of Tryptic soya broth (TSB) and incubated at 37°C for 48 h. The contents of the tubes were removed and then stained with 0.25% safranin. An adherent film on the surface of the glass tube was taken as evidence of slime formation. The absence of a film or the mere presence of a ring at the liquid-air interface was interpreted as a negative result (.). In the study, positive results were recorded as strong (+++), moderate (++), weak (+). Each test was interpreted by two different observers [21].

Black Tea (*Camellia sinensis*) Extraction

Alwazah brand black tea leaves were bought from a local retail market in Baghdad and then ground to powder using a coffee blender.
The ethanol extract was prepared by mixing 50.0gm of dry powder of plant leaves added to 95% ethanol at final volume 100ml and kept at room temperature for 3 days in a round bottom flask with occasional shaking. After 3 days period, the extract was then filtered through a muslin cloth for coarse residue and finally filtration was done through whatman No.1 filter paper then sterilized by membrane filter unit 0.4µ and finally stored in airtight bottle at 4°C until use [22 and 23]. Then the concentrations 100, 200, 300 and 400 mg/ml was prepared by mixing known volume from the stock solution with ethanol 95% using the following equation:
$V1\times C1= V2\times C2$
V1= Volume that obtained from stock solution, V2= final volume, C1= Stock solution concentration, C2= final concentration.

Antibacterial Activity of the tea extract

Antibacterial activity of the tea extract was tested on the selected organism by Agar well diffusion method [10]. In this test,(0.1) ml of a 24h broth culture of bacteria adjusted to 10^8 CFU/ml (0.5 McFarland) was aseptically introduced and evenly spread using sterile "L" rod on the surface of sterile Mueller Hinton agar plates. Four wells of about 8 mm diameter were aseptically cut on agar-plate using a sterile cork borer. Fixed volumes (0.6 ml) of each concentration 100,200,300, and 400 mg/ml were then introduced into each well with the help of a micropipette. A control well was made in the centre with the extracting solvent. The plates were incubated overnight at 37°C and the diameter of any resulting zones of inhibition was measured in (millimeters) .This was repeated three times. The minimum inhibitory concentration is defined as the lowest concentration of extract that gives limited bacterial growth.

Minimum Inhibitory Concentration Determination (MIC)

The MIC of crude extracts was determined by broth macro-dilution assay. A set of test tubes with different concentrations of plant extract (100,200,300, 400 and 500 mg/ml) with the same volume were prepared. Tubes were inoculated with the test microorganism of 10^8CFU/ml (0.5McFarland standard). After incubation, tubes were examined for changes in turbidity as an indicator of growth. The first test tube that appeared clear was considered as MIC of the extract [24].

Effects of Extract on Bacterial Viable Count

Bacterial suspensions were diluted $1:10^6$ with normal saline. Equal volumes (1 ml) of the dilution and the minimum inhibitory concentration 400 mg/ml of black tea extract were mixed and incubated at 37°C during intervals 0,2,4,6 and 24 hours At every consecutive time, 0.1 ml of each mixture was spread onto two separate nutrient agar plate then incubated overnight at 37°C. The mean number of colonies was enumerated and compared with that of a control Phosphate Buffer Saline (PBS) along with the measurement of optical density

(O.D) by the spectrophotometer to show decrease in absorbance peak. This experiment was repeated two times for each combination of extract and bacterial strain [25].

Effects of extract on bacterial virulence factors and adhesion

This was assayed by mixing the extracts with solid/liquid culture media [26]. The MIC 400 mg/ml of the extract was prepared in its extracting solvent. Bacterial isolates were activated and adjusted to inoculums adjusted to 0.5 Mc-Farland standards. Blood agar, skimmed milk agar plates were prepared and the requiring extract concentrations were added to each, mixed and left to solidify. (0.1 ml) of bacterial culture was distributed on the media surface by using sterile swabs. All plates were incubated at 37°C for 18-24 hours to observe growth. While for adherence to smooth surfaces, 10 ml of each plant extract was added to test tubes and mixed with tryptic soya bacterial suspension and safranin stain was added after decanting the components to observe the adherent layer [21].

Synergistic activity of tea and antibiotics

Antibiotic discs were soaked in leaves extract for few minutes. Bacterial cultures were swabbed on their respective plates Mueller Hinton agar plates. The soaked disc was placed in the petriplate and incubated and synergistic activity was noted by measuring the inhibition zone around the discs [10].

Results and Discussion

Isolation and Identification

A total of one-hundred samples (corneal scrapings) were obtained. The material was inoculated onto MacConkey agar and Blood agar respectively. The large flat colonies that produced zones of beta-haemolysis with a grape like odor on the latter media and the colorless colonies on MacConkey agar were cultured onto Cetramide agar. On this media, colonies were yellow-blue/green pigmented. Therefore, we can assume from the corresponding results that these isolates might belong to *Pseudomonas* species. The ability to produce green-blue/yellow pigments when cultured on cetramide agar because it is a selective differential medium used for the identification of *P.aeruginosa* due to cetramide which acts as a detergent and inhibits the growth of other microorganisms figure 1. The iron content of this medium stimulates the production of pyocyanin and a fluorescent yellow-green pigment by this organism [27].

The colonies were sub-cultured onto nutrient agar for purity checking.A number of biochemical tests as shown in table 1 were performed in order to confirm the identification of the pure colonies.

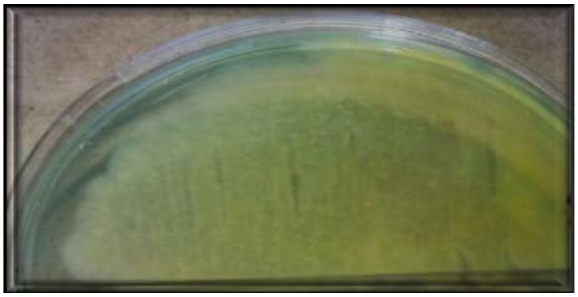

Figure 1- Pseudomonas aeruginosa growth on cetramide agar with production of pyocyanin pigment

The tested organisms had a positive result for oxidase,catalase,urease, motility,growth at 42° C and simmon's cirate utilization tests while negative for indole, Methyl red Vogas-Proskauer (MR-VP),ONPG disc and an alkaline slant and butt for triple sugar iron slants [28].

Table 1-The Biochemical Tests of Corneal Isolates

Biochemical Tests	Results
Oxidase	+
Catalase	+
Indole	-
Methyl red Vogas-Proskauer	-
Simmon's Citrate Utilization	+
Triple Sugar Iron Fermentation	- K/K
Urease	+
Motility	+
Oxidation-fermentation of Glucose	+
ONPG disc	-
Growth at 42°C	+
Haemolysis	Beta haemolysis
MacConkey	Lactose non fermenting
Cetramide	Yellow to Green Colonies

+ = Positive, - = Negative , K =alkaline

The tests confirm that the isolates belonged to *Pseduomonas aeruginosa*. Approximately, 77% of the cases were due to contact lens wear followed by 15 % trauma and 8% with unknown history. The resultant isolates identified as *Pseudomonas aeruginosa* were 30% (23/77 CL) and 25% (2/8 Unknown). This isolation percentage was higher than Hasan [29] since it was only 12% but it equals the isolation percentage stated by Bourcier [30] which was thirty percent. This agrees with most bacterial keratitis researches for the contact lens wear as the major predisposing factor followed by trauma especially in tropical countries and more with farmer workers.

Antibiotic Sensitivity test

On the other hand, the Kirby-Bauer antibiotic sensitivity assay showed that 100% of the isolates were sensitive to Neomycin, Gentamicin and Amikacin. While 91.6% were sensitive to Carbenicillin and Ceftriaxone; 66.6% were sensitive to Cefotaxime and 0% were sensitive to Tertacycline (Resistant) as shown in Figure 3.

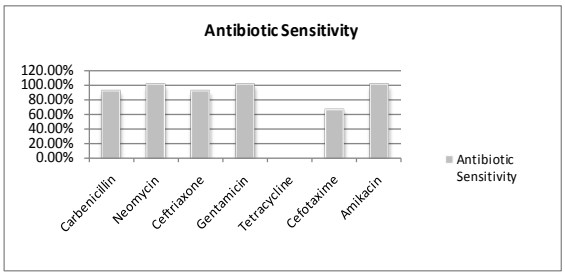

Figure 3- The Antibiotic Sensitivity test

In a related concern, only two isolates were multi-drug resistant to tetracycline, cefotaxime,gentamicin and carbenicillin table 2

Table 2- The Multi-drug Resistant Isolates

Isolate No.	PY	N	CRO	CN	TE	CTX	Ak
***P.aeruginosa*1**	**S**	**S**	**S**	**S**	**R**	**R**	**S**
P.aeruginosa2	**R**	**S**	**R**	**S**	**R**	**R**	**S**

PY=Carbenicillin, N= Neomycin, CRO= Ceftriaxone, CN= Gentamicin, TE= Tetracycline, CTX= Cefotaxime, AK= Amikacin

Smitha *et al*. [17] showed that *Pseudomonas* from keratitis isolates is most susceptible to gentamicin, ciprofloxacin and ofloxacin. Gentamicin has been found to be highly effective against *Pseudomonas* spp.

Ciprofloxacin, a second generation fluoroquinolone, also appears to be effective in treatment of *Pseudomonas* keratitis. A similar study conducted in India also showed that ciprofloxacin is highly effective against gram negative pathogens like *Pseudomonas* spp.

The *P. aeruginosa* is known for its multidrug resistance. Due to the efflux pump, *P.aeruginosa* can be resistant to antibiotics such as penicillin, cephalosporin, tetracycline and more, even without the R plasmid that is usually responsible for antibiotic resistance among bacteria. Resistance in *P.aeruginosa* is caused by the outer membrane of the bacterium, because it is not very permeable. The efflux pump is located in the cell membrane. The pump transports the antibiotics to the outer membrane of the bacterial cell [31]. However, reports on emergence of resistance to fluoroquinolones have recently been published with special emphasis to *Pseudomonas* spp. [7].

Protease was detected on skimmed milk agar by observing distinct clearing of the milk around the colony [20]. Hemolysin production was detected previous during isolation by observing beta-haemolysis around the colonies on Blood agar plates.

Adhesion was detected by the two methods:

1) On congo red agar, slime layer was indicated by black colonies on the agar surface figure 2.

2) For the tube adherence method an adherent film on the surface of the glass tube was taken as an evidence of slime formation. The absence of a film or the mere presence of a ring at the liquid-air interface was interpreted as a negative result [21]. Positive results were recorded as strong (+++), moderate (++), weak (+). As shown in Figure2; 8.33% were recorded as strong(+++), 41.66% were moderate (++) while 50% were weak (+).

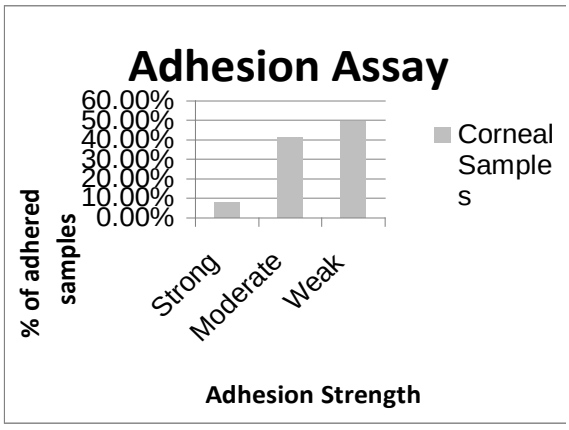

Figure 2- Interpretation of Adhesion Assay

Willicox [32] has stated that infection and inflammation during contact lens wear is often associated with microbial contamination of lenses. Several different types of microbes that colonize lenses can lead to infection and inflammation, but the most common cause of infection (microbial keratitis) remains the Gram-negative bacterium *Pseudomonas aeruginosa*. *P. aeruginosa* has a battery of cell-associated and extracellular virulence factors it can use to initiate and maintain infection. Its ability to produce proteases, to either invade or kill corneal cells, and to coordinate expression of virulence factors via quorum-sensing have been shown to be important during MK. Another important factor that contributes to the destruction of the cornea during MK is excessive activation of the host defense system.

On the other hand, the resistance to commercial antimicrobial drugs commonly used in the treatment of infectious diseases has increased due to their in discriminated use. This situation forced scientists to search for new anti-microbial and anti-inflammatory substances from various sources, such as medicinal plants.

This research includes the use of black tea extract as one of a kind study regarding this field.

Our results showed that the ethanol extracts of black tea plant had good antimicrobial coverage against *Pseudmonas aeruginosa*. The agar-well diffusion method is shown in figure 3.

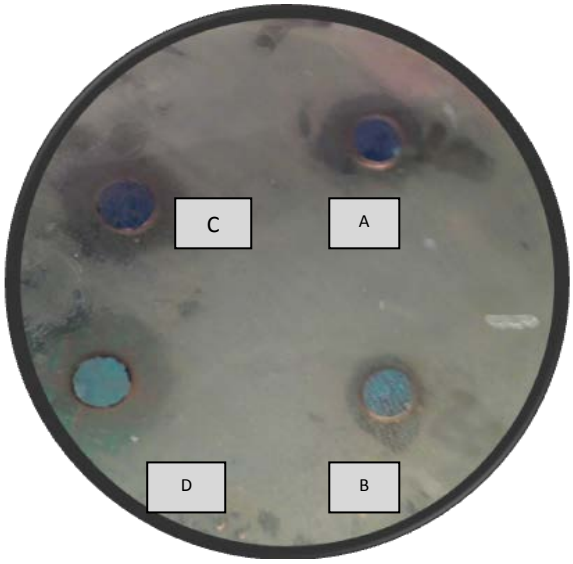

Figure 3- The agar-well diffusion method; A= 100mg/ml; B=200mg/ml; C=300 mg/ml and D= 400mg/ml

The agar well diffusion method showed that the MIC 400mg/ml of ethanol black tea extract against the bacterium *P.aeruginosa* with inhibition zone of 20 mm table 3

Table 3- The Agar-well diffusion method with diameter of inhibition zones in (mm)

Concentration of Tea Extract	Diameter of inhibition zone (mm)
100 mg/ml	13 mm
200 mg/ml	15mm
300 mg/ml	17mm
400 mg/ml	20mm

Zone diameters (in millimeters) of <5and ≥5 <10 and ≥ 10 are defined weak, moderate and good inhibitory effects respectively [33].

Tea is reported to contain nearly 4000 bioactive compounds of which one third is contributed to polyphenols. Polyphenols are bonded benzene rings with multiple hydroxyl groups. Tea polyphenols show antibacterial activity, however, it is not determined precisely which species are inhibited by antioxidants. For example, polyphenols can inhibit *clostridia* and *Helicobacter pylori* but are ineffective against intestinal lactic bacteria. The use of natural antioxidants as preservative is of great demand but it depends upon the safety and efficacy to

minimize foodborne microorganisms A number of bacteria like *Staphylococcus aureus, Vibrio cholerae, Campylobacter jejuni, Stapylococcus epidermidis* and *Vibrio mimicus* are sensitive to polyphenols but it was shown that Gram positive bacteria are more sensitive than Gram negative and antibacterial activity was apparent after one hour of incubation [34].

Ocular pathogenic bacteria that infect the eyes produce high amounts of gelatinases. (epigallocatechin gallate) ECGC present in black tea inhibited gelatinase activity produced by several strains of ocular pathogens. The inhibition can delay the invasive spread of the bacteria in the eyes that thrive on a gelatin substrate [35].

Archana and Abraham [10] showed in their study that the leaves extract of *Camellia sinensis* indicates the presence of potent antibacterial activity, which confirms its use against infection Disk diffusion method did not produce recordable results for all the three types of tea used in their study against the pathogens. Among these the methanolic extract of fresh green tea exhibited greater antimicrobial activity. The methanol extracts of the test plant produced larger zones of inhibition against the bacteria. These observations may be attributed to green tea catechin compounds and polyphenols. The organisms found to be sensitive to fresh green tea extracts were *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Candida albicans* and *Pseudomonas aeruginosa* .The fresh green tea extract with methanol was found to have high antimicrobial activity followed by commercial green tea leaves and the least activity was found in dust tea.

Kumar *et al.* [22] suggests that antimicrobial activities of tea extracts are very selective. This difference in their activity depends upon the concentration and type of the extracts. These effects may also differ depending on the bacterial species so that they may be either growth inhibitory or stimulatory [36]. The highest antimicrobial activity of tea is due to presence of catechins and polyphenoles which damages bacterial cell membrane. The green sorts of tea have shown higher antimicrobial activity than the black ones. This difference in results is probably due to presence of different contents of active substance in these tea sorts [37].Tea constituents also possess antibacterial, antiviral action, anticarcinogenic and anti mutagenic properties. However, the biologically active compounds of plants extracts are considered as antimicrobial agents, because of their ability to bind with adhesions and to disturb the availability of inhibitory effects of aqueous, methanolic and ethanolic extracts of tea plant. Minimum antibacterial activities of the aqueous were reported against most of bacteria isolated in the study. Methanolic and ethanolic extracts have shown significant zones of inhibition against bacteria that were isolated in their study [22].

In the spread plate technique, the ethanol black tea extract MIC 400mg/ml showed a visible decrease in bacterial viable count to $<5\times10^6$ CFU/ml within 24hours of incubation as shown in table 4.

Table 4- Effect of Tea Extract on Bacterial Viability

Tea extract	Number of bacteria CFU/ml at different intervals			
400 mg/ml	0hr	3h	6h	24h
	25×10^6	23×10^6	22×10^6	5×10^6

Toda *et al.* [25] showed that black tea decreased the bacterial viable count for *Staphylococcus aureus* and *Vibrio parahaemolyticus*. While Tiwari *et al.* [38] showed a successive decrease in bacterial viable count as the exposure time of *Salmonella Typhimurium* DT 98 to aqueous black tea extract.

Black tea extract also had the ability to completely inhibit *Pseudomonas* growth on blood agar by observing alpha-haemolysis (no hameolysis) in the corresponding MIC. It also inhibited skimmed milk agar digestion and production of protease enzyme. For *S.Typhimurium*, the addition of aqueous black tea extract to the growth medium at higher concentrations >6% inhibited haemolysin production [38].

While for adhesion test, black tea extract showed a mild absence of a film or the mere presence of a ring at the liquid-air interface on the surface of the glass tube figure 4.

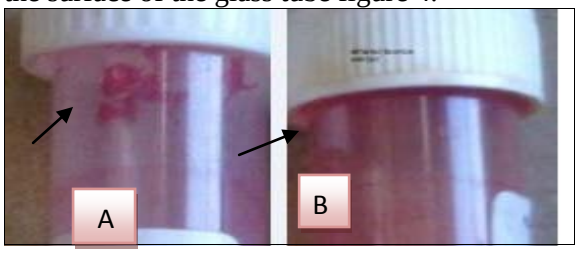

Figure 4 - Christensen's method(Tube adherence test). A= without black tea extract; B=with black tea extract

Similarly, the differences in Congo red binding were also seen in bacterial cell suspensions cultured in growth media that contained tea extract figure 5.

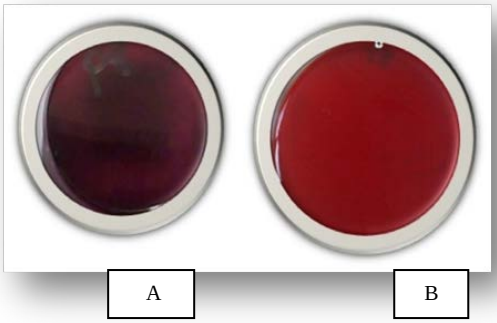

Figure 5- Congo red method. A= shows formation of black colonies on congo red agar while B=shows inhibition **of black colonies by the action of black tea extract.**

Synergistic Activity of tea and antibiotics

Combination of black tea extract with antibiotics such as Tetracycline, Carbenicillin and Cefotaxime. Results of this study showed that the sensitivity of the bacterium *P.aeruginosa* to these antibiotics has increased in presence of black tea extract Table 5.

Table 5 -Values of sensitivity of isolate *P8* to some antibiotics without and in presence of black tea extract (400 mg/ml)

Antibiotic	Inhibition zone without tea(mm)	Inhibition zone in presence of tea (mm)
Carbenicillin	18 (S)	18 (S)
Cefotaxime	13 (R)	15 (S)
Tetracycline	8 (R)	20 (S)

Tiwari *et al*. [36] showed in their study that *S. dysenteriae* was found to be more susceptible to growth inhibition by chloramphenicol, gentamycin, methicillin and nalidixic acid as the zones of inhibition were wider on nutrient agar plates supplemented with boiled black tea extract as compared to the zones of inhibition on nutrient agar plates without tea extract. Synergistic microbial growth inhibition by black tea extract and antibiotics could be attributed to the presence of dual binding sites on the bacterial surface for antibiotic and tea extract.

Conclusions

The findings discussed above shows how the results correlate with each other in many aspects. The contact lens wear still remains a complicated case every year with the patients' lack of knowledge and absence of good compliance. Black tea used in this study had a significant impact on the virulence factors produced by *P. aeruginosa* and on its resistance to some antibiotics. This indicates that this compound is a strong candidate for the treatment of corneal infections.

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