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The Biostatistical Technique to Diagnose the Extract Effect of the Black Tea (*Camellia sinensis L.*) on the *Staphylococcus aureus* Isolated from Conjunctivitis

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Abstract
This study aimed to identify of the Biostatistical technique to diagnosis the effect of extract of the black tea (*Camellia sinensis L.*) on the *staphylococcus aureus* isolated from conjunctivitis. Out of 90 eye swabs were collected from patients with conjunctivitis, 87 (96.67%) were positive for bacterial pathogens, while 3 (3.45%) were bacteriologically sterile. *Staphylococcus aureus* 28 (32.18%) had the highest prevalence, followed by *Staph. epidermides* 23 (26.44%), *Klebsiella pneumoniae* 19 (21.84%) *Streptococcus pneumonia*e 4 (4.59%) and *Moraxella Catarrhalis* 3 (3.45%). Gentamycin (89.28 %) appeared as the most potent antibiotic against *Staph. aureus*, followed by Erythromycin (64.28%), Chloramphenicol (60.71%), Azithromycin (57.14%), Clarithromycin (57.14%), Amoxicillin (42.85%) showed moderate sensitivity against *Staph. aureus*, Tetracycline (17.85%) was resisted by *staph. aureus* Gentamycin is the favors antibiotic used. the inhibitory effect of watery black tea extract on 28 *Staph. aureus* in which cooling extract show no effect in all concentrates used while heating watery tea extract show no effect on concentration (0 and 5) mg/ml, but gave (4.3, 64.3 and 96.4) % in the concentration (12.5 , 25 , 50) respectively. These result show the inhibitory effect the heating watery tea extract increase with raised of concentration.

1. Introduction
Conjunctivitis may be infectious, caused by micro-organism or non-infectious; which may be allergy caused by drug and devices such as hard and soft contact lens**.** Conjunctivitis is an inflammation of the conjunctiva characterized by cellular infiltration and exudation. The exudates may be purulent, mucopurulent, foamy, pseudo-membranous or catarrhal. Conjunctivitis in adults are probably due to viral infection, but children are more likely to develop bacterial conjunctivitis than they are viral forms.
Black tea has many more components than green tea, because of the oxidation processes that occur during ‘‘fermentation’’. Further re-actions take place when the dried finished tea leaves are extracted into water, increasing the complexity of the chemical mix in a cup of tea. Tea is the most widely drunk beverage in the world, and has been known traditionally as a healthy drink with some beneficial effects on health.

2. Methodology
2.1. The Study Objects
The study aims to achieving the following objectives
a. Determine the incidence of Staph. aureus in conjunctivitis as causative agent and its susceptibility to some antibiotic.
b. Investigate the antimicrobial (inhibitory) activity of water black tea extract against Staph. aureus .
c. Compare between antibiotic activity and inhibitory effects of tea extract on Staph. aureus isolates.
2.2. The Study Hypothesis
To achieve the study objectives, it has been putting one hypothesis as a null form (H_0), as follows:
H_0: There are no statistically significant differences at the significant level (α = 0.05), between the response's means of the patients, about the treating by (Antibiotics, and the Tea extract).

3. Theoretical Part & Literature Review
3.1. Conjunctivitis
Conjunctivitis is an inflammation of the conjunctiva characterized by cellular infiltration and exudation. The exudates may be purulent, mucopurulent, foamy, pseudo-membranous or catarrhal. Conjunctivitis may be infectious; caused by micro-organism or non-infectious; which may be allergy caused by drug and devices such as hard and soft contact lens**.** Conjunctivitis in adults are probably due to viral infection, but children are more likely to develop bacterial conjunctivitis than they are viral forms (Yamazaki, et al., 2003**)**. The types of conjunctivitis include:
a. Bacterial conjunctivitis
The most common type of infective conjunctivitis is bacterial conjunctivitis and about 90% or more of the

reported cases of infective conjunctivitis are of bacterial origin in which *Staph. aureus* is one of them (Nadi, et al., 2007). Conjunctivitis due to bacteria differs from infection due to viruses, as it is more likely to affect only one eye, and the amount of discharge and lid swelling is usually greater. Acute bacterial conjunctivitis is caused by *Staph. aureus*, *Streptococcus pneumoniae*, and *Haemophilus* species.

b. Viral conjunctivitis

A wide variety of viruses can cause conjunctivitis. Many of these infections are mild, transient, and self-limiting. Some causes of viral conjunctivitis can have significant symptoms. The most common viruses associated with conjunctivitis are adenovirus and herpes virus (Adeyeba, et al., 2010).

c. Mechanical Conjunctivitis

Mechanical irritation of the conjunctival surface can result in secondary conjunctivitis. Common causes of mechanical conjunctivitis include eyelashes, sutures, foreign bodies, and conjunctival concretions (Adeyeba, et al., 2010).

d. Traumatic Conjunctivitis

Conjunctival trauma, either direct injury (e.g., abrasions, lacerations, or epithelial defects) or indirect trauma (e.g., chemical injury) may result in the clinical manifestations of conjunctivitis (Nadi, et al., 2007).

e. Toxic Conjunctivitis

Toxic conjunctivitis may occur following the administration of drugs or exposure to noxious chemicals.

f. Neonatal Conjunctivitis

Ophthalmia neonatorum is a name for conjunctivitis that occurs within the first month of life caused by exposure of the neonatal conjunctivae to the cervico-vaginal exudates of infected women during delivery.. Among many different causes of neonatal conjunctivitis, the common etiologic agents are chemical, Chlamydia, bacterial (e.g., *Neisseria gonorrhoeae*), and herpetic.

3.2. Staphylococcus aureus

Staph .aureus is the most common bacterial cause of ocular infections. Staphylococcus which is a constant inhabitant of the skin and most membrane including the conjunctiva. *Staph. aureus* is the most important human pathogen of Staphylococci which belong to the family Micrococcaceae. It is Gram-positive, spherical, cocci which divide incompletely in three perpendicular planes to form pairs, tetrads, short chains, and bunches of grapes . Staphylococci are non-motile, non-spore-forming, occasionally capsulate. Most are catalase positive, and oxidase negative (Chart, 2007).

Morphological and cultural characters of *staph. aureus* are approximately 1µm in diameter, In liquid media, singles, pairs and short chains are also seen. On blood or nutrient agar it forms colonies 1-3 mm in diameter, colonies are smooth, low convex, glistening, opaque, sometime surrounded by a narrow zone of haemolysis on blood agar, depending on the strains. Older colonies being translucent and sticky. *Staph. aureus* is tolerant of concentrations of sodium chloride that inhibit most other bacteria, and on mannitol salt agar it forms 1 mm diameter yellowish colonies surrounded by yellow medium due to acid formation (Harvy, et al., 2007).

Staph. aureus contains protein A, (an antiphagocytic virulence factor) covalently incorporated into its cell wall, specific phospholypase C, Most strains also contain 'clumping factor' (bound coagulase) on their outer surface, which binds to fibrinogen, thus causing the organisms to aggregate in plasma. Another ('free') coagulase causes clotting of plasma in a tube test, and distinguishes this species from other human staphylococci, other enzymes include nucleases, hyaluronidase, proteinase, phosphatase, fibrinolysin (staphylokinase) (Chart, 2007).

Toxins include enterotoxins A-E, toxic shock syndrome toxin (TSST-1), epidermolytic toxins A and B, haemolysins α, β, γ, δ, and leucocidin *Staph. aureus* can cause both superficial and deep pyogenic infections as well as a number of toxin-mediated illnesses.

3.3. Black Tea (Camellia sinensis L.)

It has been increasing interest in effective plant-derived antimicrobial compounds, including those present in tea leaves (*Camellia sinensis* L.). Tea is the most widely drunk beverage in the world. Green tea, popular in the Far East, differs from the black tea familiar in the West in that an oxidation step (called ‘‘fermentation’’) occurs in the processing of the black tea (Stagg, 1980). Tea has been known traditionally as a healthy drink with some beneficial effects on health. However, current studies have revealed the biological effects of tea, such as antitumor and antimicrobial effects, even at the molecular level. Commercial teas are usually classified into three major categories:

a. Unfermented containing catechins.

b. Fully fermented black tea containing catechins (C), theaflavins (TF), and polymeric thearubigins.

c. Semifermented usually black oolong, containing both catechins and theaflavins

3.4. Chemical Composition of Black Tea

Black tea has many more components than green tea, because of the oxidation processes that occur during ‘‘fermentation.’’ Further reactions take place when the dried finished tea leaves are extracted into water, increasing the complexity of the chemical mix in a cup of tea. In addition, further chemical changes occur when a cup of tea is left to stand (Friedman, 2007).

a. Polyphenolic compounds: such as catechins. Catechins The simplest compounds in this class, the larger molecules include theaflavins and thearubigins, which are oxidation and polymerization products of simple isoflavanoids. Theaflavins, found predominantly in black tea, contain a unique seven membered aromatic ring . About 5% of the dry weight of black tea and its aqueous extracts is made up of catechins,which are simple, well-characterized isoflavanoids. These mainly consist of four compounds, (2)- epicatechin (EC), (2)-epigallocatechin (EGC), (2)- epicatechin gallate (ECG), and (2)- epigallocatechin gallate (EGCG), that may be present at concentrations of up to 1 mg/ml in a cup of tea. EGCg has anti-carcinogenic, antioxidant, as well as antimicrobial activities (Sakanaka, et al., 1992) & (Hamillton, 1995).
Immunomodulatory effect of (EGCg potently stimulates the production of interleukin-1-alpha &IL-1-beta, and tumor necrosis factor-alpha (TNF-alpha) EGCg protects against ultraviolet (UV) radiation-induced immunosuppression as well as antimicrobial activities (Sakanaka, et al., 1992).
Theaflavins, which mainly consist of four compounds, is a group of polyphenol pigment formed during the fermentation of black tea. It consist mainly of four major compounds, theaflavin (TF1), theaflavin-2-gallate (TF2A), theaflavin-3-gallate (TF3B), and theaflavin-3, 3-digallate (TF3).
Theaflavins have antioxidant, antipathogenic substance and cancer suppressor. Furthermore, theaflavins has been used to prevent coronary heart disease and to treat diabetes in clinical (Wany & Li, 2006). Morever tea polyphenols in the diet were more effective than when in the drinking water. Such result may be caused by the longer stay of diet in the mouth in comparison with the drinking water (Sakanaka, et al., 1992).
b. Caffeine: about 50 mg of which is present in a cup of black tea; caffeine is known to have ‘‘stimulant and anti-soporific actions, that elevate mood, decrease fatigue and increase capacity for work’’ (Rall,T.W.1990).
c. *Tannin:* The complex of oxidized polyphenols in tea. Tea tannins are not harmful, tea does not contain tannic acid (Wang & Li, 2006).
d. Flavonols: Leaf tea also contains small amounts of flavonols, such asquercitin, kaempferol, and myricetin (Kirk, and Othmer, 1980).
e. Flavonoid: commercial tea leaves provide a rich source of dietary flavonoids. The flavonols quercitin, kaempferol, and myricetin showed activity against gram-positive bacteria and phytopathogenic fungi in a screening test (Al-Gammal & Mansour, 1986).

3.5. Biological Activity of Tea

a. Nonmicrobiological Effects

Tea has been shown to have a wide range of beneficial physiological and pharmacological effects. Among these are slowing the catabolism of catecholamines, strengthening capillaries (‘‘vitamin P effect’’), exerting an anti-inflammatory effect by enhancing the effectiveness of ascorbic acid , acting as an antioxidant, inhibiting angiotensin-converting enzyme, having a hypocholesterolemic action (lowering of plasma cholesterol levels), activation of leukocytes in various ways, antioxidant and antimutagenic activities, and protection from the effects of radiation (Uchida, et al., 1992).

b. Microbiological activity of tea extract

The average MIC values (in µg/mL; the lower the value the greater the activity) of tea polyphenols (catechins and theaflavins) against *Staph. aureus* (192 ± 91) were much lower than the corresponding values against the genus Salmonella (795 ± 590) and *E. coli* (1519 ± 949). Although tea extracts were active against *Staph. aureus* in vitro, they were inactive against these organisms in beef as well as against *E. coli* O157:H7in vitro. It also implies that binding of flavonoids to meat proteins may prevent them from interacting with the bacteria (Kim, et al., 2004).
In one of the earliest reports, an army surgeon recommended the use of tea in soldiers’ water bottles as a prophylactic against typhoid. A series of studies, mainly from Japan, now suggests that tea extracts show several useful antimicrobial effects. Toda, et al. (1991) found that extracts of tea inhibited and killed *Staph. aureus*, *Staph. epidermidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Shigella flexneri*, *Shigella dysenteriae*, and *Vibrio* spp., including *Vibrio cholerae*. Tea extracts prevented rotavirus and enterovirus from infecting monkey kidney cells in tissue culture; this was ascribed to interference with viral adsorption rather than adirect antiviral effect and the thermostable direct hemolysin of *Vibrio parahaemolyticus* against rabbit erythrocytes (Okubo, et al., 1989).

3.6. Antimicrobial Effects of Tea Extract on Staphylococcus aureus

Staph. aureus is a highly pathogenic, Gram-positive, aerobic, toxin-producing, food borne organism that can contaminate food and infect the skin, lung, heart, and other organs. The bacterium causes food borne diseases worldwide. Toda, et al., (1991) later reported that tea at concentrations identical to those found in the beverage (a ‘‘cup’’ of tea contains ca. 3 mg of solids per ml) inhibited methicillin-resistant *Staph. aureus*. Extracts of black and green tea inhibited the hemolytic activities of staphylococcal alphatoxin (Stewart, 2005)..
A tea extract, ECGC, or theaflavin digallate inhibited the growth of methicillin-resistant *Staph. aureus* strains in culture. Higher levels (MIC = 800 mg/mL) were needed to inhibit Gram-negative rods (*E. coli*, *Klebsiella pneumoniae*, *Sa.typhi*, *Pr. mirabilis*, *Ps. aeruginosa*, *and Se. marcescens*) compared to MIC concentrations of

50–100 µg/mL of EGCG against several strains of Staphylococci.
The authors suggest that the structure of the cell wall as well as the variable affinities of ECGC to cell wall components (peptidoglycans) may govern the various susceptibilities of Gram-positive and Gram-negative bacteria to EGCG.
These observations show that tea catechins, theaflavins, and tea extracts containing both classes of polyphenolic compounds exhibited strong antibacterial activities against pathogenic bacteria that cause infectious illnesses in humans (Yoda, et al., 2004).
Pathogenic bacteria that infect the eyes produce high amounts of gelatinizes. ECGC inhibited gelatinize activity produced by several strains of ocular pathogens with an IC50 value of ~200 µM. The inhibition can delay the invasive spread of the bacteria in the eyes that thrive on a gelatin substrate (Blanco, et al., 2003).

3.7. Synergy of Combinations of Flavonoids (catechins) and Medical Antibiotics

The beneficial effects of combinations of flavonoids and medicinal antibiotics minimizing any side effects and arresting or delaying development of antibiotic resistance:
a. Aqueous tea extracts inhibited methicillin-resistant *Staph.aureus* as well as a wide range of nonresistant pathogenic organisms. ECG converted a methicillin-resistant phenotype to a methicillin-sensitive one.
b. Combinations of EGCG and carbapenem antibiotics exhibited synergistic activities against clinical isolates of methicillin-resistant *Staph. aureus* (Hu, et al., 2002).
c. EGCG synergizes the activity of β-lactam antibiotics against *Staph. aureus* by binding to the peptidoglycan component of the bacterial cellwall (Zhao, et al., 2001).
d. Theasinensin A, a decomposition product of EGCG, prevented antibiotic resistance of methicillin-resistant *Staph. aureu*s.
e. ECG was more effective in modulating β-lactam antibiotic resistance in *Staph. aureus* than ECGC .Nongalloylated catechins also potentiated the activity of oxacillin against *Staph. aureu*s (Stapleton, et al., 2004).
f. EGCG at doses MIC values of <100 mg/mL reversed resistance of Staphylococci to tetracycline. The beneficial effect of EGCG at the cellular-molecular level appears to be due to increased intracellular retention of tetracycline and may be associated with the inhibition of the expression of efflux pump proteins.
g. A combination of catechins and the antibiotic ciprofloxacin acted synergistically to alleviate chronic bacterial prostatitis in rat (Lee, et al., 2005).

3.8. Antibiotic susceptibility

Methicillin-resistant *Staph. aureus* (MRSA) strains are now very common in hospitals and the community, therefore alternative antibiotics are used. These include vancomycin, teicoplanin, rifampicin, gentamicin, clindamycin, fusidic acid or erythromycin. In severe life-threatening infections combinations of antibiotics are often used. Vancomycin is the most effective and widely used antibiotic against severe cases of MRSA infections. However, vancomycin-resistant *Staph. aureus* (VRSA) strains have also been isolated from patients (Trans, 2007).
Staph. aureus quickly developed resistance to early antibiotics. In 1942, the introduction of benzylpenicillin (penicillin G) temporarily addressed staphylococcal infections, but continued use caused the selection of resistant strains that produced penicillinase (β-lactamase). Kirby first described penicillinase-producing strains of *Staph. aureus* in 1944, and most hospital isolates were resistant to penicillin within a few years. There are a wide variety of β-lactamases that hydrolytically inactivate β-lactam antibiotics like penicillin; β-lactamases can be either plasmid or chromosomally mediated (Shanmuganathan, et al., 2005).
By 1948, the prevalence of resistant strains had seriously reduced the value of benzylpenicillin in the treatment of *Staph. aureus* infections and by the end of the 1950s, *Staph. aureus* had acquired resistance to virtually all available systemic antibiotics, including erythromycin, streptomycin, and the tetracyclines. During the 1950's chloramphenicol was widely used clinically was accepted as a promising broad spectrum antibiotics, but for recent years there was multiresistant *Staph. aureus* and also the reported cases of aplastic anemia and childhood leukemia after prolonged use of chloramphenicol (Kaye, et al., 2004).

4. Materials and Methods

4.1. Equipments

The types of equipments are shown in Table 1. as follows:

Table 1. The Types of Equipments

Type of Equipment	Manufacture (Origin)
Autoclave	Arnold and Sons (USA)
Distillator	GFL (Germany)
Digital camera	Sony (Japan)
Electric oven	Memmert
Incubator	Memmert
Light microscope	Olympus (Japan)
Micropipette	Oxford (USA)
PH-meter	LKB (Sweden)
Standard loop 0.01 ml	Hemedia (India)
Sensitive balance	Memmert
Refrigerator	Ishtar (Iraq)
Water bath	Memmert

4.2. Culture Media

Culture media (solid and broth) were prepared according to the manufacturer company for each of them and when pH adjusted the media sterilized in autoclave at 121°C for 15 minutes except sugar fermentation medium.

1. Blood Agar Medium (Oxoid) And Chocolate Agar.
2. MacConkey Agar Medium (Himedia - India).
3. Mannitol Salt Agar Medium (Himedia - India).
4. Nutrient Agar Medium (Himedia - India).
5. Müeller Hinton Agar Medium (Himedia - India).
6. Gelatin Agar Medium (Himedia - India).
7. Motility Test Medium (Himedia - India).
8. Simmon Citrate Medium (Himedia - India).
9. Pepton Water Medium (Bio Life - Italy).
10. Christensen's Urea Medium (Bio Life - Italy).
11. (MR - VP) Medium (Himedia - India).
12. Sugar Fermentation Medium (Himedia - India).

4.3. Reagent and Solution

All reagents prepared according to Baron & Finegold (1990), Collee, et al., (1996) and Macfaddin (2000):

1. Catalase Reagent (H_2O_2 30%)
2. Oxidase Reagent (N-N-N-N tetramethyl parapheylene diaminsdihydrochlored) **(**Mast-UK**)**
3. Covocs Reagent (Mast-UK**)**
4. Methyl Red Reagent (Mast-UK**)**
5. Voges - Proskaur Reagent (Mast-UK**)**
6. Macfarland Solution (Tube 0.5**):**

It has been used to compare the turbidity of bacterial suspension to obtain an approximate cell density of **$1.5x10^8$** cell/ml.

7. Physiological Saline (Dilution - Solution**):**

It has been prepared by dissolving 9 mg of NaCL in 1000 ml distilled Water.

8. Gram Stain solutions:

It has been prepared as described by MacFaddin (2000).

9. Antibiotic Disc:

Table 2. show the types of antibiotics as follows:

Table 2. The Types of Antibiotics and it's Content

Antibiotic	Abbreviation	Content mg / dick
Amoxicillin	AMO	20 mg
Azithromycin	AZM	15 mg
Clarithromycin	CLR	15 mg
Chloramphencol	C	30 mg
Erythromycin	E	15 mg
Gentamycin	CN	10 mg
Tetracycline	TE	30 mg

4.4. Methods

A. Patient, Specimens collection and Culture

A total of 90 samples (eye swabs) were collected from 90 out patients with conjunctivitis during the period July–September (2010), attending ophthalmology clinic of AL-Hakiem hospital in AL-Najaf governorate of both sexes with a different age groups.

Each swab was inculcated onto Mannitol salt agar, Blood agar, Chocolate agar, Nutrient agar and MacConkey agar at **37**°C for 24 – 48 hours , while the Chocolate agar plates were incubated in the presence of CO_2 for 24 hour at **37**°C .

B. Identification of Bacteria

The isolated bacteria identify according to he diagnostic procedures recommended by Macfaddin (2000) and Washingtoun, et al., (2006).

1. Microscopy Examination

It included the examination of shape, gram stain reaction, arrangement of cells and flagella.

2. Catalase Test

With a loop, transferred small amount of pure growth from the agar on the surface of a clean, dry glass slide, then a drop of 30% H_2O_2 was placed onto a portion of a colony on the slide, the evolution of bubbles of gas, indicates a positive test.

3. Oxidase test

A filter paper circle was placed into a sterile plastic disposable Petri-dish and moistened with several drops of the freshly prepared oxidase reagent (1%), then, a small portion of the colony to be tested was removed and rubbed on the moistened filter paper, then observation of a color change to blue or purple within 10 seconds indicated a positive result.

4. Sugar Fermentation Test

Tubes containing sugar broth were inoculated from 24 hour. Old bacteria culture and incubated at **37**^{o}C for **3** days. The positive result was detected by a change in the color of broth from red to yellow, with or without the appearance of air bubble in Durham tube.

5. Urease Production Test

A slant of christenen's medium was inoculated with the colony of tested organism, incubated at **37**^{o}C, and examined after 4 hours and 24 hour. Urea-splitting organisms were identified by the change of the color from yellow to purple-pink.

6. Gelatine Hydrolysis

Gelatin agar medium was inoculated with the colony of tested organism, incubated for **3-5** days in **37** ^{o}C. Then, the plates flooded in solution of Frazier regent for **5 -10** minutes. Gelatin-liquefying organisms were identified by the present of clear zone around the tested colony.

7. Hemolysis Production test

Blood agar medium was streaked with a pure culture of bacteria and incubated at **37** ^{o}C for **24-48** hour. The appearance of a clear zone, surrounding the colony is an indicator, of β-hemolysis while the presence of green-color indicate α-hemolysis.

8, Motility Test

Tubes containing motility test medium were stabbed once at the center with an inoculating needle then, incubated at **35**^{o}C for **24-48** hour. Motile bacteria spread out from the line of inoculating; but non motile bacteria grows only a long the stab line while surrounding medium remains clear.

9. Coagulase Test

a. Slide Test for Bound Coagulase (clumping factor)

A drop of lyophilized human plasma was placed on a clean, dry glass slide. A drop of DW was placed next to the drop of plasma as a control. By a sterile loop, an amount of the isolated colony was emulsified with each drop. When clumping was observed in the coagulase plasma drop and a smooth homogeneous in the control, the result was recorded positively. Clumping in both drops indicate the organism autoagglutinates.

b. Tube Test for Free Coagulase

Lyophilized human plasma in 0.5 ml amounts was placed in glass tube, and a visible portion of growth from isolated colonies was emulsified in the plasma by rubbing the material on the side of the tube while holding the tube at an angle, then the suspension was incubated for **1-4** hours at **37**^{o}C, the presence of clot that can not be resuspended by gentile, shaking, was recorded as a positive result. Organism that fails to clot the plasma with in 24 hour is considered coagulase negative.

10. Api-staph

This test was doing according to the manufacture company(Bio Merieux-France).

C. Antimicrobial Sensitivity Test (kirby - Bauer Method)

Two-ml of brain heart infusion broth have been inoculated with an isolated colony of test bacteria and incubated for **24** hour at **37** ^{o}C. After that, the turbidity of bacterial suspension has been adjusted turbidity of McFarland

(0.5) standard tube. 0.1 ml of bacterial suspension has been spread on the surface of Mueller Hinton medium plate and left to dry; Antimicrobial disks have been placed and incubated for **24** hour at **37**^{o}C (Bauer, et al., 1966). The resulting zone of inhibition have been measured by using a ruler and compared with zones of inhibition determined by CLSI (Clinical laboratory standards institute, 2008) and to decide the susceptibility of bacteria to antimicrobial agent , whether being resistant or susceptible.

D. Watery Tea Extract Preparation (AL- Barari)

Black tea (*Camellia sinensis* L.) were purchased directly fro shop. It was fully grinded by blender and then the cold watery extract was prepared by 10 gm of blended tea were weighted and placed into 500 ml flask contained 200 ml of distilled water, it was stirred, for 15 minute. Next using filter paper and filter funnel filtered it. The filtrates was used as stock solution (5%) or equal 50 g/ml and it used for prepare the following concentrates (0, 5, 12.5, 25, 50). Hot watery extract was prepared at the same steps except the replacement cooling distilled water by heating distilled water.

E. Antibacterial Activity of Tea Extract in Wells

This test was doing by using Agar diffusion method in wells (Egroore , 1985) in which each *Staph. aureus* isolates inoculated in Müeller Hinton Agar and then five wells (5mm) diameter were made in each plate. 0.2 ml of each concentration put in each well and incubated in **37**°C at 24 hour. The inhibition zone were measured for each concentrate.

5. The Results and Discussion

5.1. Bacteria Isolates

From 90 eye swabs examined, 87 (96.66%) were positive for bacterial pathogense, while 3 (3.45%) were bacteriologically sterile. The results of the most primary important characteristic and tests required for the isolation and identification of these 87 isolates are shown in Table 3.

Table 3. Biochemical tests used for the diagnosis of bacterial isolates

	Gram Stain	Catalase	Oxidase	Urease	Gelatinase	Glucose	Lactose	Motility	Hemo -lysin
Staph. Aureus	+	+	_		+			_	+
Staph. Epidermides	+	+	–		–			–	–
Strep. Pneumonia	+	–	–					–	+
Moraxella catarrhalis	–	+	+	–	–	–	–	–	V
Klebsiella pneumonia	–	+	–	+	–	+	+	–	–

V: It means Variable

Also, Table 4. shows the total distribution of bacterial pathogens isolated. *Staph. aureus* 28 (32.18%) had the highest prevalence, followed by *Staph. epidermides* 23 (26.44%), *Klebsiella pneumoniae* 19 (21.84%) *Streptococcus pneumonia*e 4 (4.59%) and *Moraxella Catarrhalis* 3 (3.45%).

This study reveals that conjunctivitis due to bacteria accounts for (96.66%) of the cases of infectious conjunctivitis in AL-Hakiem hospital during the period of July-September (2010). These findings accords well with that of Adeyeba, et al., (2010) who reported that about 75.2% or more of the reported cases of infections conjunctivitis is a common disease phenomenon in communities due to climatic factors and unhygienic behaviors.

Staph. aureus (32.18%) was the most prevalent organism isolated in this study. This is in agreement with the work of Locather – khorazo, et al., (1967) who reported that two-thirds of ocular infections were due to *Staph. aureus*. The predisposing factors to staphylococcal conjunctivitis include pollution and poor diet while overcrowding poor hygiene and poor ventilation help in the spread of the disease. The organism can be introduced to the eye through contact, for mites, dust and rubbing of the eye with contamination finger.

Table 4. Distribution of bacterial pathogens isolated from eye (conjunctival) swabs

Organisms	No. of isolates	Percent (%)
Staphylococcus aureus	28	32.18
Staphylococcus epidermidis	23	26.44
Klebsiella epidermidis	19	21.84
Streptococcus pneumoniae	4	4.59
Moraxella caltarrhalis	3	3.45
Total	**87**	**96.67 %**
-	**90 eye swabs examined**	

5.2. Antibiotic susceptibility of Staphylococcus aureus

Table 5. shows the antibiotic susceptibility test of *staph. aureus* isolates from conjunctival swabs in which Gentamycin (89.29%) appeared as the most potent antibiotic, followed by Erythromycin (64.29%), Chloramphenicol (60.71%), Azithromycin (57.14%) Clarithromycin (57.14%), Amoxicillin(42.86%) showed moderate sensitivity against *Staph. aureus*, Tetracycline (17.86%) was resisted by *staph. aureus* Gentamycin is the favours antibiotic used. Gentamycin has be used with caution because of its toxigenicity to the kidney nephron as reported by Greenwood, (1998).

Erythromycin could therefore, be used for the management of infection if other first line drugs are not available. Chloramphenicol (60.71%) in this study has slightly sensitive due to of indiscriminate use of this antibiotic.

Table 5. Antibacterial (inhibitory) effect of the watery Black Tea extract

Types of Antibiotics	Sensitivity		Resistance	
	No.	%	No.	%
Gentamycin	25	89.29	3	10.71
Erythromycin	18	64.29	10	35.71
Chloramphencol	17	60.71	11	39.29
Azithromycin	16	57.14	12	42.86
Clarithromycin	16	57.14	12	42.86
Amoxicillin	12	42.86	16	57.14
Tetracycline	5	17.86	23	82.14

5.3. Test the Study Hypothesis

H_0: There are no statistically significant differences at the significant level (α = 0.05), between the response's means of the patients, about the treating activity by (Antibiotics, and the Tea extract).

To test the study hypothesis validity, **Kendall's W Test** was used as showed in table (6):

Table 6. Results of the Kendall's W Test

Treating activity	Kendall's W	Chi-Square (χ^2)	df.	Mean Rank	Sig.
Antibiotic activity	0.184	1.286	1	1.71	0.257
Tea extract				1.29	

[Critical value of Chi-Square (χ^2) at (df. = 1), and (α = 0.05)] = 3.841

The results in Table 6, refers to there were no exist statistically significant differences at the significant level (α = 0.05), between the response's means of the patients, about the treating by (Antibiotic activity, and the Tea extract). Which is supported by the calculated value of Chi-Square (1.286) is less than the critical value of Chi-Square (3.841) at the significant level (α = 0.05), and the sig. (p-value) (0.257) is greater than (α = 0.05). This means that will be accepted the null hypothesis (H_0).

5.4. Inhibitory Effect of Watery Black Tea Extract

Table 7. illustrate the inhibitory effect of watery black tea extract on 28 *Staph. aureus* in which cooling water extract show no effect in all concentrates used while heating watery tea extract show no effect on concentration (0 and 5) mg/ml and (14.3, 64..3 and 96.4) % in the concentration (12.5 , 25 , 50) respectively. These result show the inhibitory effect the heating watery tea extract increase with raised of concentration.

Table 7. The Antibiotic susceptibility of 28 isolates of *Staphylococcus aureus* isolated from Conjunctiva swabs

Black Tea Extract Concentration mg/ml	Inhibition (%)	
	Cooling Water Extract	Heating Water Extract
0	0	0
5	0	0
12.5	0	14.3%
25	0	64.3 %
50	0	96.4 %

6. Recommendations

a. Study the inhibitory effect of watery black tea extract on *Staph .aureus* and other pathogenic bacteria.

b. Determine a presumptive connection between green or black tea consumption and lower risk of infection to humans.

c. Define additive and/or synergistic activities of mixtures of flavonoids with other plant-derived antimicrobials.

d. Evaluate the effectiveness of tea flavonoids against antibiotic-resistant food borne pathogens.

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