Assessment of Antibacterial Activity of Green Tea
Against Bacterial Eye Infection in Shendi Town

Submitted in partial fulfillment of the degree of MSc. In Medical Laboratory Science (Microbiology).

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بسم الله الرحمن الرحيم

قَالَ تَعَالَى: (وَالآرَضَ مُحَدَّنَاهَا وَالقِينَا فِيهَا رَوَاسِي وَأَنْبِتْنَا فِيهَا مِنْ كُلِّ شَيْءٍ مُّوزُونٍ) 

صِدَاقَ اللَّهِ الْعَظِيمِ

الْجَبْرِ الأَلْبَىٰ "١٩"
Dedication

I dedicate this work to …

Soul of my great parents …

To My sisters and brothers

To My all friends

With my love
Acknowledgment

First of all my thanks to ALMIGHTY ALLAH for giving me health and strength to accomplish this work.

Secondly special thanks to my director and supervisor Dr. Ahmed Mohammed Ahmed loka for his encouragement, follow up and guiding and directing.

My thanks and gratitude's must also go to Us. Khalid saed who generosity offered me unlimited help in this study.

My thanks and appreciation is also extended to mainly………………

Last, but not least I should thank and appreciate any one who helped us directly or indirectly in the preparation and revision of this study during the research work or writing and analyzing the manuscript. My best regards to all without any exceptions.
ABSTRACT

descriptive, hospital based prospective study. done during period from March to August 2018. The aim of study was assess and identify. The samples were collected from patients of eye infection by sterile swab from Almak Nemer out patients. The laboratory work was done in Microbiology Department, Faculty of Medical Laboratory Sciences Shendi University. The bacterial isolation was done by culture on blood Agar and identification was confirmed by gram stain and biochemical tests such as coagulase, catalase and following slandered guideline. Anti microbial activity of crude extract of green tea was tested against the isolated bacteria. Using cup plate method by using different conc 12.5-25- 50% and 100%. The bacterial inhibition zone was increased by concentration. The study concluded that the green tea have useful agent as antimicrobial activity against bacteria that cause eye infection such as S. aureus, S. pneumonia and P. aeruginosa.
دراسة وصفية جريت قسم الاحياء المختبرات الطبية جامعة شندي في الفترة من مارس الى يونيو 2018، وكان الهدف من ذلك تحدد أثر مستخلص الشاي الأخضر في اللتهاب العيون البكتريه في مدنية شندي. اخذت العينات من المرضى والمصابين باللتهابات العيون، اخذت العينات بقطن معقم من مستشفى المك نمر، ثم ارسلت العينات إلى قسم الاحياء الدقيقة في كلية المختبرات جامعة شندي. البكتريات المعزوله تمت زراعتها على اجار الدم وتم التعرف عليها بصبغه غرام والتفاعلات الكيميائية caulase and catalase …etc. ثم

مستخلص الشاي تم استخدامه على البكترياء المعزوله، باستخدام حفر طبق الاجر، بمختلف التركيز وهم كتالي:

100%, 50%, 25%, 12.5%, 5%, 2.5%, 1%

معدل تثبيط النمو يزيد بزيادة التركيز.

من خلال هذه الدراسة نوصى بإن الشاي الاخضر يستخدم كمضاد حيوي للتهابات العيون البكتريه مثل S.aureus, S.pneumonia and P.auruogenos
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CHAPTER ONE

1. INTRODUCTION

1.1 Introduction

The recent recognition of emerging infectious diseases has heightened appreciation of the importance of epidemiologic information. In addition, some well-known pathogens have assumed new epidemiologic importance by virtue of acquired antimicrobial resistance (e.g., Penicillin-resistant *Pneumococcal*, Vancomycin-resistant *Enterococci*, and multi-resistant *Mycobacterium tuberculosis*). (RAY et.al, 2004).

The ability to direct therapy specifically at a disease-causing infectious agent is unique to the management of infectious diseases. Its initial success depends on exploiting differences between our own makeup and metabolism and that of the microorganism in question the mode of action of antimicrobials on bacteria (RAY et.al, 2004).

Some antibiotics have become almost obsolete because of drug resistance consequently new drugs must be sought for, so herbal treatment is one possible way to treat diseases caused by multidrug resistant bacteria. The use of plant extracts and phytochemicals with known antibacterial properties may be of immense importance in therapeutic treatments. In the past few years, number of studies has been conducted in different countries to prove such efficiency (Indranil et al., 2006). In recent years, the health benefits of consuming green tea, including the prevention of cancer and cardiovascular diseases, the anti-inflammatory, antiarthritic, antibacterial, antiangiogenic, antioxidative, antiviral, neuroprotective, and cholesterol-lowering effects of green tea and isolated green tea constituents are under investigation. However, adding green tea to the diet may cause other serious health concerns (Sabu, et al., 2010). Tea is
one of the most popular beverages consumed worldwide. Tea, from the plant Camellia sinensis, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea (Cabrera, 2006). The association between tea consumption, especially green tea, and human health has long been appreciated (Weisburger, 2000; Sato, et al., 2000). Bacterial eye infection has worldwide distribution, affecting persons of all ages, races, social strata and both genders. The present study aimed to isolate and identify the common bacteria causing eye infection. Symptoms: red, pain, watery eyes, light sensitivity, swelling around the eyes and the most common types of bacteria that cause eye infection STAPHYLOCOCCUS aureus.

The tea {Camellia sinensis} tea leaves contain thousands of chemical compounds when they are processed these compounds break down in group of reaction called {oxidation} prevention:- good hygiene and especially hand washing is essential before touching any area eye infection treatments :- with early diagnosis and treatment. Antibiotic resistance is a problem that plagues even common place eye infections according to a new comprehensive study published Thursday Ophthalmology Analyzing more than 3,000 bacterial samples over a five year period diligently (Namita, et al. 2012).
2.1. Rationale:

Utilization of plants for Eye healing purposes is getting popular as they are believed to be beneficial and free of side effect. Although conventional antimicrobial drugs are available but increase resistant to this drug can result in treatment failure.

The aim of this study has been conducted for the effectiveness of Camellia sinensis leaves to eliminate the bacteria which can be used as preventive or treatment agent for eye infection.

To verify the claimed activity of this plants use to treat Eye infections, this study was designed to answer this question.
1.3. Objectives

2.1. General objective

To detect antibacterial activity of green tea against bacterial caused eye infections.

2.1.2. Specific objectives

1. To isolate the bacteria that causes eye infections.

2. To detect antibacterial activity of green tea extract against bacteria isolated from eye.

3. To determine minimum inhibitory concentration (MIC), of crude green tea extract.
CHAPTER TWO
2. LITERATURE REVIEW

Conjunctivitis is a common infection usually caused by bacteria or virus. It is very contagious and often affect children. We are a highly visual species. Most of our information about the world comes to us through our eye, and most of our cultural and intellectual heritage is stored and transmitted as words and images to which our vision is there for one path to better understanding ourselves and as [8].

Your eyes can become infected by a variety of different viruses, fungi, and bacteria. Each contaminant causes different afflictions, but eye infections typically show signs of irritation or pain, redness, or inflammation [9].

Because of increasing antibiotic resistance, herbal teas are the most popular natural alternatives for the treatment of infectious disease and are currently gaining more importance. We examined the antimicrobial activities of 31 herbal teas both alone and in combination with antibiotics [10].

Protected inside the orbit, most eye structures are not exposed to the outside environment. The eyelid (eyelash, conjunctiva, and cornea) is an immunologic defense mechanism of the eye: the surface of the eye is exposed to hostile environmental array. Anatomic adaption and immunologic defense has involved to protect the human eye [24].

(Bony orbit, eye lid prevent entry of organism foreign substance)

Tear from lacrimal gland wash away organisms. Immunoglobulin (secretary age, bactericidal substance eye infection caused by the four major group. Normal flora of the eye plays an important role in maintaining ocular homeostasis by various
mechanisms. They comprise of mainly bacteria which do not cause infection in normal conditions but can be a main source of infection after ocular surgery or trauma, immune compromised[11].

2-1 Medicinal plants against human pathogen: Diseases are the major cause of death in the developing countries and counts human to 50% of it. Anti microbial agents are essentially important in reducing the global burden of infectious diseases. However, resistant pathogen develop and spread—the effectiveness of the antibiotic is diminished. Bacterial keratitis is an infection and inflammation of cornea that cause pain, reduced vision, light sensitivity and tearing or discharge from the eye that can in severe cases loss of vision. Bacterial keratitis progresses rapidly and corneal destruction may be complete in 24-48 hours with some of the more virulent bacterial. Suppurative keratitis is serious problem in all tropical countries.

Information comes to us through our eyes. Our vision. The eye is made of three layers enclosing various anatomical structures: the outermost layer known as the fibrous tunic it composed of the cornea and sclera, the middle layer, known as the vascular tunic conjunctiva which run to the edge of cornea. The conjunctiva also covers the moist black surface of eye lids and eye ball. The structures and functions of the eye are complex. Each eye constantly adjusts the amount of light it let in focuses on objects near and far and produces continuous images to the brain.

The microbial flora of patients with acute bacterial conjunctivitis, corneal ulceration, blepharitis, dacryocystitis and discharging has been investigated staphylococcus aureus, streptococcus pneumonia and homophiles influenza were the main isolated. Antibiotic was effective against pathogens.

The medicinal value and benefits of traditional plants were recognized since ancient times. In Africa and other developing countries, these traditional
medicines derived from plants have continued to form the basis of rural medical care. This is due to the fact that these medicine are easy to get hold of them and available in cheap prices. Traditional medicine, in one form or another is widespread throughout the world. As its name implies, it is part of the tradition of each country, where it is handed down from on. Its acceptance by population is largely conditioned by cultural factors and much of traditional medicine therefore may not be readily transferred from one country to another.

2.1 Tea:

2.1.1 Scientific Classification:

Kingdom: Planate.
Order: Ericales.
Family: Theaceae.
Genus: Camellia.
Species: C. sinensis.
Binomial name: Camellia sinensis (L.) Kuntze. (Namita et al., 2012).

2.1.2 Description:

Tea is one of the most widely consumed beverages in the world, second only to water, and its medicinal properties have been widely explored. The tea plant, Camellia sinensis, is a member of the Theaceae family, and black, oolong, and green tea are produced from its leaves. It is an evergreen shrub or tree and can grow to heights of 30 feet, but is usually pruned to 2-5 feet for cultivation (Foster, 2000). The leaves are dark green, alternate and oval, with serrated edges, and the blossoms are white, fragrant, and appear in clusters or singly (Foster, 2000). Tea is the second most commonly drank liquid on earth after water. It is being consumed socially and habitually by people since 3000 BC. The pleasing astringent taste and refreshing boost it provides is so deep-pervasive that its potential health benefits and medicinal properties are often over looked.
Ongoing scientific exploration points that the certain potential health benefits derived from tea have important implications on human health (Sharangi, 2009).

**Tea, various types with varying properties:**

**Green tea:**

It is prepared from unfermented leaves compared to the leaves of oolong tea which are partially fermented and black tea which are fully fermented. Green tea is rich in varieties of beneficial chemicals with maximum positive effects on human beings. (Sharangi, 2009)

2.1.3. Green Tea Composition:

Green tea chemical composition is complex: proteins (15–20% dry weight) whose enzymes constitute an important fraction; aminoacids (1–4% dry weight) such as Teanine or 5-Nethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, lysine; carbohydrates (5–7% dry weight) such as cellulose, pectins, glucose, fructose, sucrose; lipids as linoleic and linolenic acids; sterols as stigmasterol; vitamins (B, C, E); xanthic bases such as caffeine and theophylline; pigments as chlorophyll and carotenoids; volatile compounds as aldehydes, alcohols, esters, lactones, hydrocarbons, etc.; minerals and trace elements (5% dry weight) such as Ca, Mg, Cr, Mn, Fe, Cu, Zn, Mo, Se, Na, P, Co, Sr, Ni, K, F and Al. Due to the great importance of the mineral presence in tea, many studies have been carried out to determine their levels in green tea leaves and their infusions (Cabrera et al., 2006).

2.1.4 Constituents:

Unlike black and oolong tea, green tea production does not involve oxidation of young tea leaves. Green tea is produced from steaming fresh leaves at high temperatures, there by inactivating the oxidizing enzymes and leaving the polyphenol content intact. The polyphenols found in tea
are more commonly known as flavanols or catechins, and comprise 30-40 percent of the extractable solids of dried green tea leaves. The main catechins in green tea are epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG), with the latter being the highest in concentration. Green tea polyphenols have demonstrated significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial properties in numerous human, animal, and in vitro studies. (Foster, 2000).

**2.1.5. Dosage and Toxicity:**

Green tea is generally considered a safe, non-toxic beverage and consumption is usually without side effects. The average cup of green tea contains from 10-50 mg of caffeine, and over-consumption may cause irritability, insomnia, nervousness, and tachycardia. Because studies on its possible teratogenic effect are inconclusive, caffeine consumption is contraindicated during pregnancy. Lactating women should also limit caffeine intake to avoid sleep disorders in infants (Foster, 2000). The dosage for green tea beverage varies, depending on the clinical situation and desired therapeutic effect. The phenol content of green tea infusion is between 50-100 mg polyphenols per cup, depending on species, harvesting variables, and brewing methods, with typical dosages ranging from 3 to 10 cups per day. Cancer preventative effects are usually associated with dosages in the higher end of the range. Green tea extracts standardized to 80-percent total polyphenols are dosed at an average of 500-1500 mg per day (Foster, 2000).

A small study in Japan demonstrated a special green tea catechin preparation (30.5% EGCG) was able to positively affect intestinal dysbiosis in nursing home patients by raising levels of Lactobacilli and Bifidobacteria while lowering levels of Enterobacteriaceae, Bacteroidaceae, and Eubacteria. Levels of pathogenic bacteria
metabolites were also decreased. An in vitro study also demonstrated green tea possesses antimicrobial activity against a variety of gram-positive and gram-negative pathogenic bacteria that cause cystitis, pyelonephritis, diarrhea, dental caries, pneumonia, and skin infections. (Foster, 2000). Theophylline in tea is used to prevent respiratory diseases like wheezing, shortness of breath, and difficulty breathing caused by asthma, chronic bronchitis, emphysema, and other lung diseases. It relaxes and opens air passages in the lungs, making it easier to breathe (Huerta et al., 2005). The tea plants extract fluoride from the soil which in turn is accumulated in its leaves. Therefore, tea is a very rich source of fluoride and one cup of tea may contain between 0.3 mg and 0.5 mg of fluoride. This has strong binding ability to enamel particles on the tooth surface that prevents dental decay (Hamilton-Miller, 2001).

2.2.1.0 Antimicrobial Properties of Tea (Camellia sinensis):

2.2.1.1 Microbiological effects:

In one of the earliest reports, an army surgeon recommended the use of tea in soldiers’ water bottles as a prophylactic against typhoid. Until recently, good evidence for a useful antimicrobial activity of tea was missing. Although there had been several reports (some anecdotal) of the antibacterial effects of tea in vitro and in vivo, mainly against intestinal pathogens, these were somewhat superficial and fragmentary (Miller, 1995). Within the past few years, this situation has changed. A series of well-conducted, systematic studies, mainly from Japan, now suggests that tea extracts show several useful antimicrobial effects, found that extracts of tea inhibited and killed Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Salmonella typhimurium, Salmonella
enteritidis, Shigella flexneri, Shigella dysenteriae, and Vibrio spp., including Vibrio cholera (Miller, 1995).

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 S. aureus. A similar finding was made with respect to Bordetella pertussis. Other workers showed that aqueous extracts of green tea inhibited cariogenic streptococci, including Streptococcus mutans; activity against other harmful mouth flora has been reported (Miller, 1995). Tea extracts prevented rotavirus and enterovirus from infecting monkey kidney cells in tissue culture; this was ascribed to interference with viral adsorption rather than a direct antiviral effect. Preventive and curative effects of tea on influenza virus have been claimed in a patent. Killing of pathogenic protozoa by tea extracts has been reported in the Russian-language literature, but it is difficult to assess the significance of this (Miller, 1995).

2.2.1.2 Microbiological activities of tea in vitro:

The polyphenol fractions of tea have been closely examined for their antimicrobial properties. Several studies have shown that purified catechin fractions from green and black tea, and ECG and EGCG in particular, inhibit the growth of many bacterial species and possess anticariogenic properties. Specifically, a commercially available preparation of tea polyphones, Sunphenon, prevented the attachment of a cariogenic S. mutans strain to hydroxyapatite and also inhibited its glucosyl transferase activity (Miller, 1995).

2.2.1.3 Microbiological activities of tea in vivo:

Work in animals suggests that tea reduces the incidence of caries. There is a report in the Japanese-language literature that drinking green tea reduced the incidence of dental caries among school children, but the
validity of the conclusions is difficult to assess. It has been suggested that this effect was due to an increased intake of fluoride, but this seems unlikely; rather, the polyphenol moiety of tea was thought to be responsible. Elvin-Lewis and Steelman claimed to have noted statistically improved dental health in children who drank at least one cup of tea daily compared with the dental health of those whose intake was less than 3 cups per week. Unfortunately, these findings are reported in abstract form only and do not appear to have been followed up. There are suggestions from the patent literature that tea catechins may have some commercial usefulness in the general field of mouth hygiene (Miller, 1995).

2.2.1.4 Mechanism of green tea to kill bacteria:
Many studies have shown EGCG to be the most effective antibacterial polyphenol at typical or slightly lower concentrations than found in regular brewed green tea. The exact mechanisms of EGCG’s antibacterial activity are unknown, but it is believed that EGCG disrupts the cell membrane and prevents DNA super coiling, ultimately leading to the destruction of the bacterial cell. In vitro experiments suggest that EGCG affects fungal pathogens, Gram-positive bacteria, and Gram-negative bacteria, but Gram-positive bacteria are particularly vulnerable to the polyphenols. The difference between Gram-positive and Gram-negative bacteria lies in the cell wall. The Gram-positive cell wall has several layers of peptidoglycans that are joined together to form a thick, rigid wall, whereas the Gram-negative cell wall has an additional membrane covering the thinner wall of peptidoglycans. This outer membrane contains lip polysaccharides and lipoproteins, which are vital to the bacteria’s survival under enormous bacterial pressure (Sace, 2008). Many such experiments have shown that catechins in green tea inhibit the growth of S. mutans and S. sobrinus with a minimum inhibitory concentration between 50 and 1000 μg/ml, and 250-500 μg/mL for P.
gingivalis. These concentrations all fall within the range of concentration found in a typical cup of tea (Taylor, et al., 2005). Other study a total 18 E.coli isolate collected from urine sample submitted to clinical microbiology laboratories of selected hospitals Urimia, Iran, the average MIC and MBC of the water soluble green tea extract against all isolates of E.coli were 122.9±40.3 mg/ml (Hoseeni N et al., 2007).

2.3.1. COMPLICATION OF EYE INFECTION:

Serious complications of eye infection include damage to the retina and the formation of scars and ulcer in the cornea that can obstruct vision. Some infections, like syphilis, can also provoke problems are often the only visible symptom of wider infections (Cheesbrough, 2006).

It used to practice for physicians to prescribe antibiotics for many types of infections from the common cold to conjunctivitis physicians some time give antibiotic before surgery to prevent infection from occurring in the first place. Doctors have also used antibiotics following injections for treating age-related macular degeneration (Taylor, et al., 2005).

All these practices, in addition to many other factors are thought to have contributed to the increase in antibiotic-resistant bacteria. More organisms become exposed to antibiotics, the more of chance that a few will mutate in such a way that they can survive the drugs attack mechanism. A 2014 study from the journal ophthalmology that looked at drug resistance over a 25-year period found that more than half of all bacteria causing endophthalmitis, one the most severe eye infections, are now resistant to the commonly prescribed antibiotic cefazolin.
Among the two most common staphylococcus species. Bacterial resistance to the drug methicillin each patient is different and has different risk factors (Taylor, et al., 2005).
CHAPTER THREE
3. MATERIAL AND METHODS

3.1. Study design
This is descriptive prospective study to determine the effect of green tea in bacterial eye infection, it is Hospital based study.

3.2. Study area
study population every patients come to ophthalmic in mak Nemer hospital and all health center .[shendi educated hospital - nurab hospital ]refer to Matama and shendi locality3.3.

Study population.
Eye infections in Shendi and Matama Hospital {Almak Nemer hospital and other hospital}.

3.3. Sample size
All patients coming with eye infections to ELmak nemer and Shendi hospital in time June to august 2018.

3.9. Methodology
3.9.1 Collection of specimens
Under aseptic conditions, Eye swabs were collected using sterile cotton swabs. The samples were transported in transport medium immediately to the laboratory for investigation.

3.9.2. Culture of specimens
Collected swabs were cultured on Blood agar (HI media) and Macconkey’s agar (HI media) plates using standard sterile loop. All plates were incubated aerobically at 37°C for 24 hrs.

3.9.3. Cultural characteristics
After the incubation period, the plates were examined for the size, color, edges, side views, odor and surface of colonies.
3.9.4. Purification and preservation of isolates
Purification was done by repeated sub-culturing of typical and well isolated colonies on nutrient agar (HI media). The resulting growth was check for purity using Gram’s staining procedure. Obtained pure cultures were presented through inoculation into nutrient agar slope and stored at 4°C after 24hrs incubation at 37°C.

3.10. Microscopic examination
Smears were made from sub cultured colonies, fixed by gentle heating and stained using Gram’s staining technique by Barrow and Feltham (1993), and then examined microscopically under oil immersion.

3.11. Identification of bacteria
Preparation of smear:

1- On clean dry slide put one drop of normal saline and by loop after sterilization take small amount of well grown single bacterial colony from the agar plate and mix it with normal saline.

2- Spread the well mixed bacteria and normal saline drop in slide in area about 1 cm.

3- Leave the slide to air dry then fix it by heating by flame by passing the slide in flame 3 times.

Gram stain:

Principle:
Differences in Gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of Gram positive and
Gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria. Retention of crystal violet by Gram positive organisms may also be due in part to the more acidic protoplasm of these organisms binding to the basic dye (helped by the iodine) (Cheesbrough, 2006).

3.12. Biochemical identification

3.12.1. Catalase test:

This test is used to differentiate those bacteria that produce the enzyme catalase from non producing bacteria.

Principle:

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is catalase positive.

Required:

Hydrogen peroxide, 3% H₂O₂ (10 volume solution).

Method:

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.

2. Using a sterile wooden stick or a glass rod (not Nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution
3. Look for immediate bubbling.

Results:

Active bubbling . . . . . . . . Positive catalase test.

No bubbles . . . . . . . . . . Negative catalase test (Cheesbrough, 2006).

3.12.2. Coagulase test

The test was used to identify *S. aureus* which was coagulase positive from other Staphylococci species which were coagulase negative. Coagulase causes plasma to clot by converting fibrinogen to fibrin. On clean slide place drop of distilled water and emulsify a colony of tested organism then add loop full of plasma on the suspensions and mixed gently the results was clumping of organisms within 10 seconds (Cheesbrough, 2006).

3.12.3. DNAase test

The test was used to differentiate *S. aureus* (positive) from other *Staphylococci* species (negative). The tested organism was culture on a medium which contain DNA, after overnight incubation the colonies were tested by flooding the plate with a weak hydrochloric acid (Hcl). The acid precipitates UN hydrolyzed DNA. DNase produced colonies were surrounded by clear area indicating DNA hydrolysis (Cheesbrough, 2006).

3.12.4. Mannitol fermentation test

This medium was used to differentiate *S. aureus* from other *Staphylococci* species. A portion of colony was inoculated on mannitol salt agar containing 75 g\l sodium chloride and incubated aerobically at 37ºC for 18-24 hrs. *S. aureus* ferment mannitol producing yellow colonies (Cheesbrough, 2006).
3.12.5. Oxidase test
Strips of filter paper was soaked in 1% solution of tetra–methyl-p-phenylene diamine dihydrochloride and dried in hot air oven and then placed on clean glass slide bacterial colony by sterile glass rod and rubbed on filter paper strip. If purple color developed with 5-10 seconds, the reaction was considered positive (Cheesbrough, 2006).

3.12.6. Indole production test
Indole production was carried out as described by Barrow and Felham (1993). The tested organism was inoculated into pepton water and incubated at 37ºC for 48 hrs. 1 ml of kovac’s reagent was run down along the side of the test tube. Appearance of a pink color in the reagent layer within a minute indicated positive reaction.

3.12.7. Citrate utilization test
It was done according to Cheesbrough (2005). Simmon’s citrate medium was inoculated with the tested organism and incubated at 37ºC for up to 48 hours; utilization of citrate was recognized by a bluish color.

3.12.8. Urease test
It was done according to Cheesbrough (2006). The slant surface of urea agar medium was streaked with the tested microorganism and incubated at 37ºC for 24- 48 hrs. The development of a pink color was indicative of production of NH₃. Negative and weak tests were left for a week before being considered as negative.

3.13. Extraction of medical plants
3.13.1. Collection and preparation of plant samples
They were authenticated by protocol of Medicinal and Aromatic plant Research Institute (MAPRI). The dried Green tea were cleaned from dust and grass; 100 grams of each plant sample were separately crushed to a powder from using sterilized mortar and pestle.
3.13.1. Preparation of plant extract:
The plant extracts were prepared using the solvent water. 15g of green tea powder were taken and homogenized with 100 ml of the distil water (Kumar A et.al, 2012). The mixture was left in hot air oven under 80ºC for 2 hours. After that leave to cool and filter the mixture by piece of gauze then by what man filter paper No.1. The extract is heated in hot air oven under 60ºC to make stock crude material. The crude is weight by sensitive balance and dissolved in known volume of sterile distilled water to obtain desired concentration.

Reagents:

Mueller–Hinton agar:

1. Mueller–Hinton agar should be prepared from a dehydrated base according to the manufacturer’s instructions. The medium should be such that control zone sizes within the published limits are produced. It is important not to overheat the medium.

2. Cool the medium to 45–50 ºC and pour into the plates. Allow to set on a level surface, to a depth of approximately 4mm. A 90 mm plate requires approximately 25 ml of medium.

3. When the agar has solidified, dry the plates for immediate use for 10 – 30 minutes at 35 ºC by placing them in the upright position in the incubator with the lids tilted.

4. Any unused plates are stored in a plastic bag, which should be sealed and placed in the refrigerator. Plates stored in this way will keep for 2weeks [18].
**Turbidity standard (0.5 McFarland standards):**

Prepare the turbidity standard by pouring 0.6 ml of a 1% (10 g/l) solution of barium chloride dehydrate into a 100 ml graduated cylinder, and filling to 100 ml with 1% (10 ml/l) sulfuric acid. The turbidity standard solution should be placed in a tube identical to the one used for the broth sample. It can be stored in the dark at room temperature for 6 months, provided it is sealed to prevent evaporation.[16]

**Swabs:**

Sterile wooden swab with applicator from Ningbo MFLAB medical instruments Co.Ltd.

**Sterile Normal saline concentration 0.85%:**

Prepared by dissolve 8.5 g of NaCl in 1000 ml of Distill water.

**Disposable plastic Petri dish:**

90 mm size disposable plastic Petri dish (Marina Co.Ltd).

**Glass porer:**

7mm diameter glass porer.

**Automatic pipette:**

- Automatic pipette variable (5 – 50 µ.l).
- Automatic pipette variable (100 – 1000 µ.l).

** Disposable plastic automatic pipette tips:**

- Blue tips (size: 1000 µ.l)
- Yellow tips (size: 200 µ.l).
Serology small glass tubes:
12 x 75mm glass test tube.

Large size glass tubes:
15 x100mm glass test tubes.

Glass Erlenmeyer flask:
500ml size flask and 250ml size flask.

glass Beaker:
300ml size.

Bacteriological loops:
- Nichrome ring loop and needle loop (HI-MEDIA).

Test tubes racks.

Autoclave (Dixon), Incubator (Thermo Scientific), Oven and Bunsen burner.

Procedure of inoculation in Mueller Hinton agar plates:

1- This growth to a tube of sterile saline and mix then Compare the tube with the turbidity standard and adjust the density of the test suspension to that of the standard by adding more bacteria or more sterile saline.

2- Touch with a loop the tops of each of 3–5 colonies, of similar appearance, of the organism to be tested.

3- Transfer Inoculate the plates by dipping a sterile swab into the inoculum. Remove excess inoculums by pressing and rotating the
swab firmly against the side of the tube above the level of the liquid.

4- Streak the swab all over the surface of the medium three times, rotating the plate through an angle of 60° after each application. Finally, pass the swab round the edge of the agar surface. Leave the inoculums to dry for a few minutes at room temperature with the lid closed.

5- By using metal porer of size 6 mm in diameter, make 5 pores in agar plate then fill the pores by green tea extract by using automatic pipette in volume 50 µ.1 of concentrations 500, 250, 125, 62.5 and 31 mg/ml.

6- Incubate the plates for 24h in incubator under aerobic condition in 37ºC.

7- The diameter of each zone (including the diameter of the disc) should be measured and recorded in mm.

8- The measurements made with a ruler on the under-surface of the plate without opening the lid.

3.14. Determination of Minimum Inhibition Concentration (MICs) by agar plate dilution method

The principle of agar plate dilution was the inhibition of the growth on the surface of the agar by the plant extracts incorporated into the medium. Plates were prepared in series of decreasing concentrations of the plant extraction in the following order 50, 25, 12.5, 6.25 mg/ml. the bottom of
each plate was marked off into 4 segments. The tested organisms were
grown in broth over night to contain $10^8$ organisms per ml. loop-full of
diluted culture was spotted with a standard loop which delivers 0.001ml
on the surface of each segment and then incubated at 37°C for 24 hours.
The end point (MIC) was the least concentration of antimicrobial agent
that completely inhibits the growth. Results were reported as the MIC in
mg/ml.

3.16. Data analysis
Data were entered, check and analyzed using Microsoft Excel 2007 and
SPSS (Statistical Package of Social Science) soft program version 11.5.
Chapter Four

4. Results

4.1. Frequency and percentage of sampling according to age group

Table 1: Frequency and percentage of sampling according to age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>15</td>
<td>43%</td>
</tr>
<tr>
<td>21-40</td>
<td>10</td>
<td>29%</td>
</tr>
<tr>
<td>41-60</td>
<td>5</td>
<td>14%</td>
</tr>
<tr>
<td>&gt;60</td>
<td>5</td>
<td>14%</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100%</td>
</tr>
</tbody>
</table>

4.2. Frequency and percentage of sampling according to gender

Table 2: Frequency and percentage of sampling according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>15</td>
<td>43</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>57</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100</td>
</tr>
</tbody>
</table>

4.3. Bacteriological Result

4.3.1. Frequency and Percentage of bacterial growth.

55 studied specimen, 30 showed bacteria growth while the remaining 6 were negative for growth (table 3).

Table 3: Frequency and Percentage of bacterial growth.

<table>
<thead>
<tr>
<th>Result of culture</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>30</td>
<td>86</td>
</tr>
<tr>
<td>No growth</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3. *Shows distribution of isolated bacteria.*

<table>
<thead>
<tr>
<th>Isolated Bacteria</th>
<th>Frequency</th>
<th>Percent %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>7</td>
<td>22.6</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>20</td>
<td>64.5</td>
</tr>
<tr>
<td><em>S. pneumonia</em></td>
<td>4</td>
<td>12.95</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>31</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4. *Shows the degree of growth inhibition of the isolated bacteria caused by aqueous extract concentration*

<table>
<thead>
<tr>
<th>Concentration Isolated Bacteria</th>
<th>%100</th>
<th>%50</th>
<th>%25</th>
<th>12.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std</td>
<td>Mean</td>
<td>Std</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>13.1</td>
<td>4.0</td>
<td>13.2</td>
<td>2.8</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>13.4</td>
<td>4.0</td>
<td>11.3</td>
<td>3.4</td>
</tr>
<tr>
<td><em>S. pneumonia</em></td>
<td>12.7</td>
<td>3.8</td>
<td>11.7</td>
<td>3.2</td>
</tr>
</tbody>
</table>

MDIZ mean diameter of growth inhibition zone in (mm), If MDIZ.

- > 7 sensitive.
- < 7 Resistant
Table 5. Antibacterial activity of green tea extracts against bacteria isolated from eye infection in different concentrations:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>S. aurous</th>
<th>S. pneumoniae</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>100%</td>
<td>20</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>50%</td>
<td>20</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>25%</td>
<td>20</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>12.5%</td>
<td>19</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>P-value</td>
<td>0.065</td>
<td></td>
<td>0.000</td>
</tr>
</tbody>
</table>
Chapter Five

5. Discussions

5.1. Discussions

This study was conducted in Elmak Nemer hospital and shendi hospital. Patients were selected from ophthalmology out patients. The lab work was done in lab of microbiology department in faculty of medicine science. 35 patients with symptom of eye infection were included in the study. 15 of them were male and 20 female in ratio 3:4. The patient age included in the study from 1 to 70 years. The specimen were culture and isolated and identified. 31 specimen were isolated with positive growth as followed. *S.aureus* was 20 (64.5%) *P. aeruginosa* and *Strepto pneumonia*.

Table (1) show the degree of growth and inhibition. The most common bacteria inhibit by extraction is *S.aureus* after that *P.aeruginosa* and *Strepto pneumonia* and table (1) show antimicrobial activity of green tea extraction against bacteria isolated from eye in different concentration.

100% - 50% - 25% - 12.5% all bacteria not grow in stock concentration. The inhibition zone is large and clear. The inhibition zone is different in different concentration.

Table 4 show that eye infection is spread in female than male and in age up to 50 years (elder).
5.2 Conclusion

On this basis study, it could be concluded that

1. the age less than 20 years is the most age group exposed to eye infection
2. the most common bacteria that cause eye infection is \textit{S.aureus}
3. the concentration 100\% is more effective than other concentration.
4. Concluded from this study that Camellia Sinesis extract had markable sensitivity toward gram positive bacteria.
5.3. Recommendation

Depending on the obtained results it could be recommended that:

1- Apply more advanced and sensitive techniques to screen for the natural anti microbial agents.

2- Determination of minimum inhibitory concentration (MIC) for active ingredient of each bacteria including in this study.

3- Patients should be advised to use the concentrated extraction of tea to wash their eyes.

4- Further studies with large to investigate the high resistant rate of diluted (extraction of green tea) in growth bacteria
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Philipn baker, (2006), obstetrics by ten teachers18:41-46….


## Appendix I

### Table (1) Standard formula and uses for some materials

<table>
<thead>
<tr>
<th>Materials</th>
<th>Standard formula</th>
<th>Gram/liter</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muller Hinton agar (HI-MEDIA)</td>
<td>-Meat, infusion solids from 300g - Casein acid hydrolysate - Starch - Agar.</td>
<td>2.0</td>
<td>Suspend 38 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>C.L.E.D Agar w/Bromo Thymol Blue (HI-MEDIA)</td>
<td>-Peptic digest of animal tissue. - Casein enzymichydrolysate. - Beep extract. - Lactose. - L-Cystine. - Bromothymol blue. - Agar.</td>
<td>4.0</td>
<td>Suspend 36 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.128</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Peptone water (HI-MEDIA)</td>
<td>-Peptic digest of animal tissue. -Sodium chloride.</td>
<td>10.0</td>
<td>Suspend 15.0 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Simmons’s citrate agar.(HI-MEDIA)</td>
<td>-Magnesium sulphate. -Ammonium dihydrogen phosphate. -Dipotassium phosphate. -Sodium citrate.</td>
<td>0.2</td>
<td>Suspend 24.28 grams in 1000 ml distilled water. Heat, to boiling, to dissolve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>Ingredients</td>
<td>Amounts</td>
<td>Preparation Notes</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>---------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Urea agar base (Christenis). (HI-MEDIA)</td>
<td>- Peptic digest of animal tissue. - Dextrose. - Sodium chloride. - Disodium phosphate. - Monopotassium phosphate. - Phenol red. - Agar.</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Nutrient agar (HI-MEDIA)</td>
<td>- Peptic digest of animal tissue. - Sodium chloride. - Beef extract. - Yeast extract. - Agar.</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mannitol salt agar (HI-MEDIA)</td>
<td>- Proteose peptone. - Meat extract. - Sodium chloride. - D-Mannitol. - Phenol red. - Agar.</td>
<td>10.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Litmus milk (HI-MEDIA)</td>
<td>- Skim milk powder. - Litmus.</td>
<td>100.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Bile esculin agar (HI-MEDIA)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>-Peptic digest of animal tissue</td>
<td>5.0</td>
<td>Suspend 64.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense into tubes or Flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.</td>
</tr>
<tr>
<td>-Beef extract</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>-Esclulin</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>-Bile salts</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>-Ferric citrate</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>-Agar</td>
<td>15.0</td>
<td></td>
</tr>
</tbody>
</table>

Biochemical reactions of some enterobacteria
Appendix I

Biochemical reactions of some staphylococci

<table>
<thead>
<tr>
<th>Bacteria name</th>
<th>Catalase</th>
<th>Coagulase</th>
<th>Mannitol</th>
<th>DNAse</th>
<th>Novobiocin</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S.epidermidis</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S.saprophyticus</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Appendix I

جامعة شندي
كلية الدراسات العليا
قسم المختبرات الطبية
تخصص أحياء دقيقة

1- Name ...........................................................................
2- Sex ..............................................................................
3- Age .............................................................................
4- Residence.....................................................................
5- Past history red eye, eye discharge itching. ....................
6- What antibiotic use response good better best ................
7- Type of infection spontaneous post .................................
8- Duration of infection ....................................................
9- If use lence or not ........................................................