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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).

By

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بسم الله الرحمن الرحيم

الآيـــــــــــة

قال تعالى:

(و الارض مددناها والقينا فيها رواسي وانبتنا
فيها من كل شيء موزون)

صدق الله العظيم

الحجر الآية "19 "

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.

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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

this is descriptive , hospital based prospective study . done during period from march to august 2018.the aim of study was asses 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 swap the isolated bacterial. 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.auruogenosa.

ملخص البحث

دراسة وصفيه جريت قسم الاحياء المختبرات الطبية جامعة شندي في الفترة من مارس الى يونيو 2018 ، وكان الهدف من ذالك تحدد اثر مستخلص الشاي الاخضر 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 الى قسم الاحياء الدقيقة في كلية المختبرات جامعة شندي. البكتريات المعزوله تمت زرعتها على اجار الدم وتم التعرف عليها بصبغه غرام والتفاعلات الكيمائية ثم caugolase and catalase ...etc.

مستخلص الشاي تم استخدامه على البكترياء المعزوله ، بأستخدام حفر طبق الاجار ، بمختلف التركيز وهم كتالي : %100%,50%,25%,12.5. معدل تثبيط النمو يزيد بزيادة التركيز .

من خلال هذه الدراسة نوصى بإن الشاي الاخضر يستخدم كمضاد حيوي للتهابات العيون البكتريية 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, swellening around the eyes and the most common types of bacteria that cause eye infection STAPHLOCOCCUS aureus.

the tea {camellia sinensis } tea leaves contain thousands of chemical compound s 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.Anti biotic resistance is a problem that plagues even common place eye infections according to a new comprehensive study published Thursday Ophthalmology Analyzing more than 3,000bacterial 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 isolates 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 common infection usually caused by bacteria or virus. It is very contiguous and often affect children We are a highly visual species .most our information about the world comes to us through our eye and most 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 affliction s but eye infection s typically show signs of irritation or pain redness or inflammation [9].

Because of increasing anti biotic 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 tea both alone and in combination with anti biotic[10]

protected in side orbit Most eye structure are not exposed to outside environment eye lid (eye lash conjunctiva and cornea) {External eye] normal flora is present in the external part of f the eye lid {in very small number} 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 organism foreign substance) Tear from lacreimal gland Washing away organism immunoglobulin {secretary age bactericidal substance eye infection caused by the four major group Normal flora of the eye plays 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 Against pathogen; Diseases are the major cause of death in the developing countries and counts human to 50% of it - aAnti microbial agent s are essentially important in reducing the global burden of infectious diseases -how ever are resistant pathogen develop and spread-the effective ness of the anti biotic is diminished . bacterial keratits 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 case 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 country Information come to us through our eyes our vision .the eye is made of three layer inclosing various anatomical structure-the outer most layer known as the fibrous tunic it composed of the cornea and sclera -the middle layer .known as the vascuiet 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 strepto coccus pneumonia and homophiles influenza were the main isolated antibiotic was effective against pathogens.

The medicinal value and benefits of traditional plants were recognized sinceient 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 ever green 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

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

It used   to   practice   for   physician s   to   prescribe   antibiotics for many   types   of infections   from   the   common   cold   to conjunctivitis   physicians some   time   give anti biotic   before surgery to   prevent   infection from   occurring   in the   first place . doctor 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   anti biotic- 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. a2014 study   from   the   journal ophthalmology .that   looked   at   drug   resistance   over   a25-year period found that   more than half   of   all   bacteria   causing endophthalmitis , one the most   severe eye infections , are now resistance   to   the commonly   - prescribed anti biotic cefazolin.

Among   the tow 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 37ºC 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 4ºC after 24hrs incubation at 37ºC.

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 forcatalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is catalase proced.

Required:

Hydrogen peroxide, 3% H_2O_2 (10 volume solution).

Method:

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

2. Using a sterile wooden stick or a glass rod (notaNichrome 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 NH_3 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 with100 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 to100ml 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 cans 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 8.5g 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 60º 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.l 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 in to 4segments. 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 37ºC 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 2007and 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

Age group Frequency Percentage

Age group	Frequency	Percentage
< 20	15	43%
21-40	10	29%
41-60	5	14%
>60	5	14%
Total	35	100%

4.2. Frequency and percentage of sampling according to gender

Table 2: Frequency and percentage of sampling according to gender

Gender	Frequency	Percentage
Male	15	43
Female	20	57
Total	35	100

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.

Result of culture	Frequency	Percentage
Growth	30	86
No growth	5	4
Total	35	100

Table 3. <u>Shows distribution of isolated bacteria</u>.

Isolated Bacteria	Frequency	Percent %
P. aeruginosa	7	22.6
S. aureus	20	64.5
S. pneumonia	4	12.95
Total	31	100.0

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

Concentration Isolated Bacteria	%100		%50		%25		12.5%	
	Mean	Std.	Mean	Std	Mean	Std	Mean	Std
P. aeruginosa	13.1	4.0	13.2	2.8	11.5	2.4	10.1	1.6
S. aureus	13.4	4.0	11.3	3.4	10.1	2.6	8.7	2.6
S.pneumoniae	12.7	3.8	11.7	3.2	9.7	1.8	9.2	1.5

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:

Concentration	S. aurous		S.pneumoniae		P. aeruginosa	
	S	R	S	R	S	R
100%	20	0	4	0	7	0
50%	20	0	4	0	7	0
25%	20	0	4	0	7	0
12.5%	19	1	4	0	7	0
P-value	0.065		0.000		0.000	

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 1to 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 strepto

Table ( ) show the degree of growth and inhibition the most common bacteria inhibit by extraction is s.aureus after that p,aeurginosa and strepto pneumonia and table ( ) show anti microbial 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   *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- futher studies with large to investigate the high resistant rate of diluted (extraction of green tea) in growth bacteria

REFERNCES

ALGEOBORY ,H.A.H 2011. Comparative study between methicllin resistance staphylococcus aurous and methillin sensitive staphylococcus aurous and detect the anti microbial effects of some plant   extract on them Msc . The department of biology, College   of Science , University of Bagdad I rage Pp 62 63.

Asian Pacific journal of Tropical Bio medicine .volume 11 ,Issue 6,june ,2018,P355-404 more…

Basic laboratory   procedures in clinical   bacteriology/J.vandepitte {et al} Book .Bib ID, 3256470.Format, book,

Cabrera, C., Artacho, R and Gimenez, R (2006) Beneficial Effects of Green Tea—A Review. *Journal of the American College of Nutrition,* Vol. 25, No. 2, p.79–99.

Chen k.h, kuang, and Hsu, W.M (2001) serration marcescens corneal ulcer as   complication orthokeratology.Am.j ophthalmol 123(2):257-258.

Cleveland clinic   journal of medicine   volume75.number7 July 2008.

Das,k Tiwari,R.k.S…and   shrivastava D,K.2010 .Techniques   for evaluation   of medicinal plant products as anti microbial agent Current method s and future trends .J.med.plant.Res.4(2)pp:104-111.

Gwendolyn R.,Burton W,PaulG (1998) microbiology for   the health science ,Lippincott &Wilkins ,7:492-493

Hamilton-Miller JMT, Shah .S (2002). *Activity of the component epicatechin gallate and analogues against methicillin-resistant Staphylococcus aureus*. J Antimicrobial Chemother. 46:847–863.

Hosseni N, Zartoshti M (2007) *evaluation of synergic effect of water soluble extracts of green tea on the activity of Ciprofloxacin in urinary isolated E.coli.* Journal of biological sciences 7 (8).p 1500- 1503.

Cheesbrough, M (2006) *District laboratory practice in tropical countries*. 2nd edition. Part 2.United States of America:Cambridge University Press.

http://www.calculator.net.[on line scientific calculator for quick calculations, along with large collection of free…

Indranil B, Soroj K, Chatterjee S, Chatterjee G, C (2006). Antibacterial Potentiality of Argemone Mexicana solvent extract *against some pathogenic bacteria. Mem, Inst.* 101(6).

Indranil B, Soroj K, Chatterjee S, Chatterjee G, C (2006). Antibacterial Potentiality of Argemone Mexicana solvent extract against some pathogenic bacteria. *Mem*, *Inst*. 101(6).

Kumar A et.al (2012). *Antibacterial activity of green tea (Camellia sinensis) extracts against various bacteria isolated from environmental sources. Recent Research in Science and Technology, 4(1): p19-23.*

Kumar A et.al, 2012. Mammalian proapototic factor chaC1 and its homologues function EMBO Rep13 (12):1095-101

Millar LK, Cox SM **(1997)**. *Urinary tract infections complicating pregnancy*. Infectious Diseases Clinics of North America, 11(1):13-26.

Miller, H **(1995)**. Mini review Antimicrobial Properties of Tea (Camellia sinensis L.).*Antimicrobial agents and chemotherapy.* Vol. 39, No. 11(11) p.2375–2377.

Miller, H. J. M **(2001).** Anti-cariogenic properties of tea (Camellia sinensis).*Journal of Medical Microbiology*, 50 (4) p.299–302.

Mohamed, I H (2003).studies on aerobic bacteria isolated from male urine in Khartoum state .MSc thesis. University of Khartoum

Namita, P et al. (2012). *Camellia Sinensis (Green Tea): A Review. Global Journal of Pharmacology.*6 (2).p. 52-59.

Philipn baker, (2006), obstetrics by ten teachers18:41-46.…

Pommerville   J.C.2007.Alcamo,s   laboratory. Fundamental s of microbiology 8th   edition Jones

RAY G.C, RYAN J.K (2004). *Sherris Medical Microbiology and introduction to infectious diseases*- 4th edition – USA –page 867-871.

Sabu M Chacko, Priya T Thambi, Ramadaan Kuttan, Ikuo Nishigak. **(2010)**. *beneficial effects of green tea*: A literature review. Chinese Medicine, 5:13.

Sanford –Smith, j (1990). Eye diseases in hot climates   2ed Butterworth .Oxford.Uk.

Sato T, Miyata G **(2000)**. *The nutraceutical benefit, part I: green tea*. Nutrition. 16:315-317.

Sharangi, A.B **(2009).** *Medicinal and therapeutic potentialities of tea (Camellia sinensis L.) – A review. Food Research International.* 42 (1) p. 529–535.

 Smitha,S.,laliththa p.prajna ,VN.and   srinivasan ,M2005.susceptibility Trends of pseudomonas species from corneal   Ulcers .

Snell.RS (.2001). Clinical Anatomy for Medical Student.9th Ed pp 723-724 A mold LondonU.k.

Vandepitte, J. et al. 2003   j vandepitte   ,jverhaegen, kngbaek,p book,s Google .com –Rohner …2003.

Weisburger JH **(2000)**. *Approaches for chronic disease prevention based on current understanding of underlying mechanisms*. Am J Clin Nutr, 71(6):1710S-1714S.

World Health Organization (2002). WHO Publication on strategy for traditional medicine.

www.medbroadcast.com/condition/getcondition/Eye-infections

Appendix I

Table (1) Standard formula and uses for some materials

Materials	Standard formula	Gram/liter	Preparation
Muller Hinton agar (HI-MEDIA)	-Meat, infusion solids from 300g -Casein acid hydrolysate. -Starch. -Agar.	2.0 17.5 1.5 17.0	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.
C.L.E.D Agar w/Bromo Thymol Blue (HI-MEDIA)	-Peptic digest of animal tissue. -Casein enzymichydrolysate. -Beef extract. -Lactose. -L-Cystine. -Bromothymol blue. -Agar.	4.0 4.0 3.0 10.0 0.128 0.02 15.0	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.
kiliglar iron agar (HI-MEDIA)	.Peptic digest of animal tissue. .Beef extract. .Yeast extract. .Protease peptone. .Lactose. .Dextrose. .Ferrous sulphate. .Sodium chloride. .Sodium thio sulphate. . Phenol red. .Agar.	15.0 3.0 3.0 5.0 10.0 1.0 0.2 5.0 0.3 0.02 15.0	Suspend 57.52 grams of dehydrated powder in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix well and distribute into tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
Peptone water (HI-MEDIA)	-Peptic digest of animal tissue. -Sodium chloride.	10.0 5.0	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.
Simmons's citrate agar.(HI-MEDIA)	-Magnesium sulphate. -Ammonium dihydrogen phosphate. -Dipotassium phosphate. -Sodium citrate.	0.2 1.0 1.0 2.0	Suspend 24.28 grams in 1000 ml distilled water. Heat, to boiling, to dissolve

	-Sodium chloride. -Bromothymol blue. -Agar.	5.0 0.08 15.0	the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.
Urea agar base (Christensis). (HI-MEDIA)	.Peptic digest of animal tissue. .Dextrose. .Sodium chloride. .Disodium phosphate. .Monopotassium phosphate. .Phenol red. .Agar.	1.0 1.0 5.0 1.2 0.8 0.012 15.0	Suspend 24.01 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 10 lbs pressure (115°C) for 20 minutes. Cool to 50°C and aseptically add 50 ml of sterile 40% Urea Solution (FD048) and mix well.
Nutrient agar (HI-MEDIA)	-Peptic digest of animal tissue. -Sodium chloride. -Beef extract. -Yeast extract. -Agar.	5.0 5.0 1.5 1.5 15.0	Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes
Mannitol salt agar (HI-MEDIA)	-Proteose peptone. -Meat extract. -Sodium chloride. -D-Mannitol. -Phenol red. -Agar.	10.0 1.0 75.0 10.0 0.025 15.0	Suspend 111.02 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 minutes
Litmus milk (HI-MEDIA)	-Skim milk powder. -Litmus.	100.0 5.0	Suspend 105 grams in 1000 ml distilled water, agitating continuously. Dispense 10 ml

			amounts into 15 x 150 mm. tubes and Sterilize by autoclaving at 15 lbs pressure (121°C) for 5 minutes.
Bile esculin agar (HI-MEDIA)	-Peptic digest of animal tissue. -Beef extract. -Esculin. -Bile salts. -Ferric citrate. -Agar.	5.0 3.0 1.0 40.0 0.5 15.0	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

Biochemical reactions of some enterobacteria

Appendix I

Biochemical reactions of some staphylococci

Bacteria name	Catalase	Coagulase	Mannitol	DNAse	Novobiocin
S.aureus	+	+	+	+	+
S.epidermidis	+	-	-	+	+
S.saprophyticus	+	-	+	-	-
Micrococcus luteus	+	-	-	-	-

Appendix I

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جامعة شندي

كلية الدراسات العليا

قسم المختبرات الطبية

تخصص أحياء دقيقة

</div>

1- Name …………………………………………………………………

2- Sex …………………………………………………………………….

3- Age ………………………………………………………………….

4- Residence……………………………………………………………..

5- Past history red eye , eye discharge itching. ………………………

6- What antibiotic use response good beter best ……………………

7- Type of infection spotenous post ……………………………

8- Duration of infection ………………………………………………

9- If use lence or not ……………………………………………………