University of Shendi

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In Vitro Susceptibility Patterns of Aqueous Extract of Green Tea on Bacteria Isolated from Pregnant Women with Urinary Tract Infection Attending Shendi Hospital.

A thesis Submitted for the fulfillment of the requirement of M.Sc. in Microbiology

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الايـــــــــة

قال تعالى:

بسم الله الرحمن الرحيم

﴿اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿1﴾ خَلَقَ الْإِنْسَانَ مِنْ عَلَق ﴿2﴾ اقْرَأْ وَرَبُّكَ الْأَكْرَم ﴿3﴾ الَّذِي عَلَّمَ بِالْقَلَم ﴿4﴾ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ﴿5﴾﴾

صدق الله العظيم

سورة العلق : الايات 1- 5

Dedication

To my wonderful parents who strongly supported me all throughout.

To my beloved sister and adorable brother.

To all those whom I always love, care and respect.

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Abbreviations:

Al	Aluminum.
AST	Antimicrobial susceptibility testing.
ATCC	American type culture collection.
ATP	Adenosine tri phosphate.
BC	Before Christ
C.L.E.D	Cystine Lactose electrolyte deficient.
Ca	Calcium.
Co	Cobalt.
Cr	Chromium.
CSU	Catheter specimen of urine.
Cu	Copper
DNA	Deoxyribonucleic acid.
E.coli	Escherichia coli.
EC	Epicatechin
ECG	Epicatechin-3-gallate
EF	Elongation factor
EGC	Epigallocatechin.
EGCG	Epigallocatechin-3-gallate.
F	Fluorine.
Fe	Ferric.
GCG	Gallocatechin-3-gallate
K	Kalium.
LDL	Low density lipoprotein.
LPS	Lipopolysaccharide.
MBC	Minimum bactericidal concentration.
MDR	Multi drug resistant.
Mg	Magnesium
MIC	Minimum inhibitory concentration.

Mn	Manganese.
Mo	Molybdenum
MRSA	Methicillin resistant staphylococcus aureus
MSA	Mannitol salt agar.
MSU	Mid stream urine.
Na	Natrium.
NCCLS	National Committee for clinical laboratory standards.
Ni	Nickel
P	Phosphorus.
pH	Potential of hydrogen.
Se	Selenium.
SPA	Suprapupic aspirate.
Sr	Strontium.
UTI	Urinary tract infection.
WBCs	White blood cells.
Zn	Zinc.

Abstract:

Background: Due to several anatomical and hormonal changes, pregnant women are more susceptible to develop Urinary tract infections (UTI). UTI is a major health problem, it has been reported among 20% of the pregnant women and it is the most common cause of admission in obstetrical wards. Finding alternative antimicrobial agents from plant extracts has received growing interest. *Camellia sinensisis* a safe, non toxic, cheap beverage that has been reported to have antimicrobial effects against various pathogenic bacteria including *E. coli*.

Objectives: This study aimed to evaluate the effectiveness of aqueous extract of *Camellia Sinensis* (Green tea) on different types of bacteria isolated from pregnant women suffering from urinary tract infection.

This was a cross-sectional and hospital based study, has been conducted at University of Shendi –faculty of medical laboratory sciences- department of microbiology, between February 2015 to February 2017.Following informed consent 191 pregnant women suffering from UTI in different trimesters and different ages were enrolled in this study.

Methodology: One hundred thirty seven bacteria were isolated, different Gram positive and Gram negative bacteria, in vitro sensitivity testing using well diffusion technique against aqueous green tea extract.

Results: The mean age of pregnant women was 25.4 ± 6.7 years. Pregnant women with UTI in different trimesters 56% were in third trimester. In the study population 76% were drinking tea during pregnancy and 99.3% were drink black tea.

The main causative agent of UTI in the study population was *S. aureus* 24.8% then *E.*

coli 21.9% followed by many other bacteria.

The largest diameter of inhibition zone appeared in Gram positive *Enterococcus fecalis* (17.6 ± 1.9 mm) .The concentrations of green leaves aqueous extract used was 500, 250, 125, 62.5 and 31 mg/ml. Minimum inhibitory concentration values of aqueous extracts of green tea on test organisms, the concentration of green tea aqueous extract able to inhibit the growth of bacteria was 48.6 mg/ml against *S.aureus* and 280.2 mg/ml against *Escherchia coli*.

Conclusion: The antimicrobial activity of crude extract was compared with that of standard antimicrobial based on the mean diameter of inhibition zone. The aqueous extract exhibited maximum relative percentage inhibition against *S.aureus* (38.2%) and minimum relative percentage inhibition against *Enterobacter cloacae* was (11.1%).

المستخلص:

المقدمة : نتيجة لوجود مجموعة من العوامل التشريحية والتغيرات الهرمونية تعتبر النساء الحوامل أكثر الفئات عرضة للإصابة بإخماجات المجاري البولية.إخماج المجاري البولية يعتبر من أكبر المشاكل الصحية شيوعا و قد أشارت التقارير أن حوالي 20% من النساء الحوامل المصابات بإخماج المجاري البولية هو السبب الأساسي في دخولهن المستشفيات .تواجد بدائل من عوامل مضادة للميكروبات مستخلصة من النبات وجد طريقا للاهتمام. نبتت كاميليا ساينسس (الشاي الأخضر) هي نبتة آمنة ، غير سامة و رخيصة الثمن للتحصل عليها و قد أثبتت التقارير أن لها فعالية مضادة للميكروبات الممرضة و خاصة بكتريا الإشريكية القولونية.

الهدف :هدفت هذه الدراسة إلى معرفة مدى تأثير المستخلص المائي لنبتة كاميليا ساينسس (الشاي الأخضر)على الأنواع المختلفة للبكتيريا المعزولة من النساء الحوامل اللاتي يعانين من إخماج المجاري البولية.

أجريت هذه الدراسة التقاطعية في جامعة شندي- كلية علوم المختبرات الطبية – قسم الأحياء المجهرية في الفترة فبراير 2015 الى فبراير 2017م . وقد تضمنت عدد 191 من النساء الحوامل اللاتي يعانين من إخماجات المجاري البولية من أعمار و مراحل حمل مختلفة.

المنهجية : مائة سبعة و ثلاثون بكتيريا معزولة مختلفة الأنواع موجبة وسالبة الجرام مسببة لإخماج المجاري البولية أختبر تأثير المستخلص المائي للشاي الأخضر عليها داخل المعمل بإستخدام تقنية الثقوب النافذة أو المنتشرة .

النتائج : متوسط أعمار النساء الحوامل في هذه الدراسة 6.7 ± 25.4سنة ،النساء الحوامل المصابات بإخماج المجاري البولية في مراحل الحمل المختلفة وجد أن حوالي 56% منهن في الثلث الأخير من مراحل الحمل. و وجد أيضا في مجتمع الدراسة أن 76% من النساء الحوامل يتناولن مشروب الشاي أثناء فترة الحمل وان 99.3% منهن يتناولن مشروب الشاي من النوع الأسود.

العامل الرئيسي المسبب لإخماج المجاري البولية في مجتمع الدراسة هي بكتريا المكورات العنقودية الذهبية بنسبة 24.8% ثم الإشريكية القولونية بنسبة 21.9% تليها الجراثيم الأخرى.

اكبر نطاق للتثبيط ظهر ضد البكتيريا موجبة الجرام الكروية المعوية (17.6 ± 1.9ملم).،.تراكيز المستخلص المائي للشاي الأخضر اللتي أستخدمت هي 62،125،250،500 و 31 ملجم / مل.أقل تركيز من المستخلص المائي للشاي الأخضر قادر على تثبيط نمو البكتيريا موجبة الجرام هو 48.6 ملجم/مل ضد المكورات العنقودية الذهبية ، و في البكتيريا سالبة الجرام كان 280.2 ملجم /مل ضد بكتيريا الإشريكية القولونية.

الاستنتاجات : نتائج فعالية الشاي الأخضر ضد الميكروبات قورن مع نوع فعال من الأدوية لإيجاد نسبة معدل التثبيط المقرب.معدل التثبيط المقرب أظهر أعلى نسبة ضد المكورات العنقودية الذهبية (32.2 %) وأقل نسبة ضد الإمعائية المذرقية (11.1%).

Chapter one

Introduction

Rationale

Objectives

1. Introduction:

In the female human subject, the urinary tract has an important relationship with the reproductive organs because of its proximity. In the non-pregnant state, the uterus lies just behind and partly over the bladder while in the pregnant state the enlarging uterus affects all the tissues of the urinary tract at various times (Mittal & Wing, 2005).

Urinary Tract Infections (UTI) has become the most common hospital-acquired infection, accounting for as many as 35 % of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients. UTI accounts for a significant part of the work load in clinical microbiology laboratories and enteric bacteria remained the most frequent cause of UTI, although the distribution of pathogens that cause UTI is changing (Epoke, *et al*., 2000).

UTI is a common health problem among pregnant women (Mittal & Wing, 2005). This usually begins in week 6 and peaks during weeks 22 to 24 of pregnancy due to a number of factors including urethral dilatation, increased bladder volume and decreased bladder tone, along with decreased urethral tone which contributes to increased urinary stasis and ureterovesical reflux and up to 70 % of pregnant women develop glycosuria, which encourages bacterial growth in the urine (Van Brummen, *et al*., 2006).UTI (perhaps if untreated) can lead to serious obstetric complications, poor maternal and perinatal outcomes e.g. intrauterine growth restriction, preeclampsia, caesarean delivery and preterm deliveries (Mazor-Dray, *et al*., 2009).Furthermore, it has been observed that asymptomatic bacteriuria can lead to cystitis and pyelonephritis (Barnick & Cardozo, 1991), which can lead to

acute respiratory distress, transient renal failure, sepsis and shock during pregnancy (Gilstrap & Ramin,2001). Screening of pregnant women for UTI can minimize it, and the associated complications (Millar & Cox, 1997). Recently various risk factors of UTI during pregnancy have been reported; perhaps these are varied according the geographical, social and biological settings (Haider, *et al*., 2010). *Escherichia coli* with its multidrug resistant strains- has been found to be the commonest cause of UTI among pregnant women (Dalzell & Lefevre, 2000; Kariuki, *et al*., 2007).

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 (Chacko, *et al*., 2010).

The health-promoting effects of green tea are mainly attributed to its polyphenol content, particularly flavanols and flavonols, which represent 30% of fresh leaf dry weight. Recently, many of the a forementioned beneficial effects of green tea were attributed to its most abundant catechin, (-)-epigallocatechin-3-gallate (EGCG). Green tea extracts are more stable than pure epigallocatechingallate, one of the major constituents of green tea, because of the presence of other antioxidant constituents in the extract. In general, herbal medicines are complex mixtures of different compounds that often act in a synergistic fashion to exert their full beneficial effect (Chacko, *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, *et al*., 2006).The association between tea consumption, especially green tea, and human health has long been appreciated (Weisburger, 2000; Sato & Miyata, 2000).Some studies have suggested an inverse association between green tea consumption and the risk of kidney stone formation (McKay & Blumberg, 2002; Ishizuk, *et al*., 2003).

1.2 Rationale:

Investigating epidemiology of UTI (prevalence, risk factors, bacterial isolates and antibiotic sensitivity) during pregnancy is fundamental for care givers and health planners to guide the expected interventions. While an extensive published literature concerning UTI during pregnancy is available from other African countries (Assefa, *et al*., 2008), there is no published data concerning UTI in pregnant Sudanese women in Shendi town. The aim of this study has been conducted at the effectiveness of *Camellia sinensis* leaves to eliminate the bacteria which can be used as preventive agent from recurrent urinary tract infection during pregnancy.

1.3 Objectives of the study:

1.3.1General objective:

To evaluate the effectiveness of aqueous extract of *Camellia Sinensis* (Green tea) on different types of bacteria isolated from pregnant women suffering from urinary tract infection.

1.3.2Specific objectives:

1- To estimate the effectiveness of aqueous extraction of green tea on Gram positive and Gram negative bacteria.

2- To determine the relative percentage inhibition of green tea aqueous extract.

3- To determine the minimum inhibitory concentration (MIC) of aqueous green tea extract.

Chapter 2

Literature Review

2. Literature Review

2.1 Urinary tract infection overview:

Urinary tract infections (UTIs) are the most common infections seen in outpatient practice (Southwick, 2007). Normal urine is sterile; therefore infection could, theoretically, be diagnosed if a single bacterium was isolated from the urinary tract. In practice, voided urine becomes contaminated in the non-sterile distal urethra. Consequently, with logarithmic bacterial proliferation rates, most individuals diagnosed with urinary infection have a bacterial count of 10^4–10^5/ml. Quantitative urine culture is, therefore, a necessity for diagnosis (McCormick, *et al*., 2008).

2.2 Pathogenesis:

2.2.1 Bacterial factors:

Bacteria generally gain entry into the urinary system by ascending the urethra into the bladder and then, in some cases, ascending the ureters to the renal parenchyma. The organism that most commonly infects the urinary tract is *Escherichia coli*, and certain strains of *E. coli* are more likely to cause a UTI (Southwick, 2007).

These strains possess advantageous virulence characteristics, including increased ability to adhere to the epithelial cells of the urethra and increased resistance to serumcidal activity and hemolysin production. *E.coli* adheres by their fimbriae or pili, distinct protein hair like structures on the bacterial surface. Pyelonephritis strains are the most adherent; cystitis strains tend to be intermediately adherent. Two types of fimbriae are important for determining whether *E. coli* causes lower

or upper tract infection. Type I fimbriae specifically adhere to mannosylated proteins on the surface of bladder epithelial cells. Bacteria that adhere by type I fimbriae can be readily detached from epithelial cells by exposing them to mannose ("mannose–sensitive"). Some strains of *E. coli* have a second type of fimbriae called P fimbriae that adhere to glycophospholipids embedded in the outer surface of the plasma membrane of uroepithelial cells (Southwick, 2007).

2.2.2.0 Host factors:

Urine contains high concentrations of urea and generally has a low pH. These conditions inhibit bacterial growth. The urine of pregnant women tends to be more suitable for bacterial growth, and patients with diabetes often have glucose in their urine, making that urine a better culture medium. These factors help to explain why pregnant women and diabetic patients have an increased incidence of UTI. Mechanical factors probably are the most important determinants for the development of UTI. Mechanical factors can be grouped into two risk categories (Southwick, 2007).

2.2.2.1 Obstruction:

The flushing mechanism of the bladder protects the host against infection of the urinary tract. When bacteria are introduced into the bladder, the organisms generally are cleared from the urine. Obstruction of urinary flow is one of the most important predisposing factors for the development of a UTI. Prostatic hypertrophy and urethral strictures can lead to bladder outlet obstruction (Southwick, 2007).

Defective bladder contraction associated with spinal cord injury also results in

poor bladder emptying. These conditions result in a significant volume of urine remaining in the bladder after voiding ("increased post-void residual"), which markedly increases the likelihood of infection. Intrarenal obstruction caused by renal calculi, polycystic kidney disease, and sickle cell disease also increases the risk of renal infection (Southwick, 2007).

2.2.2.2 Urethra Length:

Women have a short urethra, which increases the risk of bacteria entering the bladder. The incidence of UTI in women (1% to 3% of women) is much higher than that in men (0.1% or less until later years). At least 10% to 20% of all women develop a symptomatic UTI at some point during their lifetime. Trauma to the urethra by sexual intercourse and the use of a diaphragm both increase the risk of UTI (Southwick, 2007).

2.3 Common Urinary Tract Pathogens:

Escherichia coli (75%), *Klebsiella* (15%), *Proteus* (5%), *Enterococci* (2%), *Staphylococcis epidermidis* (2%), *Group B streptococci* (2%) and *Pseudomonas* (rare). (Southwick, 2007).

2.4 Clinical Manifestations:

Patients with cystitis usually experience acute-onset dysuria (pain, tingling, or burning in the perineal area during or just after urination). Dysuria results from inflammation of the urethra. In addition, patients need to urinate frequently, because inflammation of the bladder results in increasing suprapubic discomfort when the bladder is distended and may cause bladder spasms that interfere with bladder distension. Some patients note blood in the urine caused by inflammatory

damage to the bladder wall (Southwick, 2007). The clinical manifestations of upper-tract disease usually overlap with those of lower-tract disease. However, in addition to symptoms of cystitis, patients with pyelonephritis are more likely to experience fever and chills, costovertebral angle pain, nausea and vomiting, and hypotension. Certain risk factors increase the likelihood of upper-tract disease (Southwick, 2007). Treatment is recommended in pregnant women because these patients are at increased risk of developing pyelonephritis. In preschool children, asymptomatic bacteriuria can result in renal scarring and interfere with normal growth of the kidneys (Southwick, 2007). Urethritis (inflammation of urethra) can be confused with cystitis. The primary symptom is burning on urination. Colony counts resulting from urine culture are less than 10^5 organisms per milliliter, and the patient usually does not experience suprapubic pain or urinary frequency. Women with vaginitis can also experience burning on urination. Therefore, in a woman with symptoms suggestive of cystitis or urethritis a companied by a vaginal discharge, (pelvic exam is warranted to exclude a pelvic infection). The physical findings associated with UTI are usually minimal. Patients with cystitis may have suprapubic tenderness (Southwick, 2007).

Patients with pyelonephritis often are febrile and may be hypotensive and have an elevated heart rate. They often are acutely ill, appearing toxic. Costovertebral angle or flank tenderness resulting from inflammation and swelling of the infected kidney may be noted. In elderly patients, pyelonephritis and gram-negative sepsis may lead to confusion and somnolence (Southwick, 2007).

2.5 Urinary tract infection in pregnancy:

Urinary tract infection (UTI) is common in pregnancy. It can be asymptomatic, as well as symptomatic, complicating the diagnostic process. It is of importance to obstetricians because of its association with significant maternal and perinatal morbidity and mortality (McCormick, *et al*., 2008)

2.5.1 Incidence:

In pregnancy, the overall incidence of UTI is approximately 8%. The incidence of asymptomatic bacteriuria in pregnant women as determined in UK studies is 2–5%.the incidence of acute cystitis is more difficult to accurately determine, as many women are treated empirically and culture not performed. However, one study over a 6-year period determined a1.3% incidence rate. The incidence of pyelonephritis during pregnancy is 2%, with up to 23% of women experiencing a recurrence in the same pregnancy (McCormick, *et al*., 2008)

2.5.2 Etiology:

Urine is bacteriostatic to most local commensal bacteria and this is thought to result from its relatively acidic pH, high osmolality and high urea concentration. In an anatomically normal urinary tract, sterility is maintained by free ante grade flow through the ureteral and urethral valves (McCormick, *et al*., 2008).

In pregnancy, significant physiological changes occur in the urogenital tract, increasing the potential for pathogenic colonization. Bladder volume increases and detrusor tone decreases. Additionally, 90% of pregnant women develop ureteric dilatation as the result of a combination of progestogenic relaxation of ureteric smooth muscle and pressure from the expanding uterus. There is relative

sparing of the left ureter because of protection from the sigmoid colon and upper rectum. The net effect, however, is increased urinary stasis, compromised ureteric valves and vesicoureteric reflux, which facilitates bacterial colonization and ascending infection (McCormick, *et al*., 2008).

Seventy percent of pregnant women develop glycosuria and this, in combination with physiological aminoaciduria of pregnancy and a fall in urine osmolality, favours bacterial proliferation. Sexual activity in women has been established as a significant risk factor for UTI. Intercourse can traumatize the urothelium of the distal urethra, resulting in increased bacterial invasion. The vagina can act as a reservoir for gastrointestinal bacteria, facilitating inoculation. In contrast with most vulval and perineal commensal bacteria, Gram negative bacteria from the bowel thrive in urine (McCormick, *et al*., 2008).

2.5.3 Classification:

Urinary tract infection in pregnancy has three principal presentations:

2.5.3.1 Asymptomatic bacteriuria:

Defined as persistent colonization of the urinary tract by significant numbers of bacteria in women without urinary symptoms (McCormick, *et al*., 2008).

2.5.3.2 Acute cystitis:

Distinguished from asymptomatic bacteriuria by the presence of symptoms such as dysuria, urgency, frequency, nocturia, haematuria and suprapubic discomfort in a febrile women with no evidence of systemic illness (McCormick, *et al*., 2008).

2.5.3.3 Pyelonephritis:

Defined as significant bacteriuria in the presence of systemic illness and symptoms such as flank or renal angle pain, pyrexia, rigor, nausea and vomiting (McCormick, *et al*., 2008).

2.5.3.4 Recurrent infection:

Urinary infection recurs in 4–5% of pregnancies. The risks of developing pyelonephritis and its potential consequences are the same as for the primary infection. The exact aetiology is uncertain but re-infection by Coliform bacteria from the vaginal reservoir can occur as a result of sexual activity. Urinary tract anomalies must be excluded and postpartum evaluation is advisable after several episodes of antenatal infection.Long-term, low dose antimicrobial cover, or single postcoital doses, have been advocated for the remainder of the pregnancy (McCormick, *et al*., 2008).

2.6. Antimicrobials in pregnancy:

Pregnancy increases glomerular filtration rates, with a resultant increase in the elimination rate of renally-excreted medications. This, combined with increased maternal plasma volume, effectively reduces serum drug concentrations and can adversely affect the amount of therapeutic activity at the target tissue (bio-availability) (McCormick, *et al*., 2008). This is especially a problem with ß-Lactam antibiotics, including penicillins and cephalosporins. In addition; polyuria and frequency reduce urinary drug concentration and the therapeutic window within the urinary tract. Consequently, it may be necessary to increase administration dosages or prescribe hydrophilic drugs to ensure efficacy

(McCormick, *et al*., 2008). It is essential to remember potential maternal side-effects, drug interactions and the possibility of teratogenicity when any medication is prescribed in the antenatal period, especially in early pregnancy (McCormick, *et al*., 2008).

2.7 Laboratory diagnosis:

Laboratory confirmation of a diagnosis of UTI is not always essential. Many female patients with uncomplicated cystitis can be treated empirically with urine samples sent for only those who fail to respond. Microbiology investigations should always be done on children, adult males, those with recurrent infections, those with risk factors for a resistant organism (recent hospitalization, residence in nursing home), and those with suspected pyelonephritis (Irving, *et al*., 2006).

2.7.1 Culture and sensitivity tests:

Urine samples are cultured using a semi-quantitative method, onto a suitable indicator agar Cystine lactose electrolyte deficient agar (CLED agar). Low numbers of organisms ($<10^3$ per ml) are ignored as urethral/perineum contaminants, unless the sample is ureteric urine or obtained via suprapupic puncture SPA. A count of 10^5 organisms per ml from an MSU is generally regarded as the significant cut-off, but pure cultures of 10^4 bacteria per ml (especially in the presence of pyuria) are also accepted as indicative of UTI (Irving, *et al*, 2006). Identification of organisms from urine, and subsequent antibiotic sensitivity tests are performed using standard laboratory methods. In some situations, identification is simply done to the genus level (e.g. 'coliform' bacillus), but it may be necessary to identify to species level (e.g. *Klebsiella*

oxytoca), particularly with isolates from inpatients on specialized units, or when multiresistant strains are found. Sensitivity tests often employ antibiotics at a higher concentration for urine isolates, compared to isolates from other specimens, reflecting the fact that many antibiotics reach the urine in high concentration following renal excretion. Direct sensitivity tests are sometimes performed on urine samples with pyuria (Irving, *et al*, 2005).

2.8 Tea:

2.8.1 Scientific Classification:

Kingdom: Plantae.

Order: Ericales.

Family: Theaceae.

Genus: *Camellia.*

Species: *C. sinensis.*

Binomial name: *Camellia sinensis* (L.) Kuntze (Namita, *et al*., 2012).

2.8.2 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).

2.8.3.0 Tea, various types with varying properties:

2.8.3.1 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).

2.8.3.2 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-*N*ethylglutamine,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).

2.8.3.3 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).

2.8.3.4 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 that 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 that 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 in 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).

2.8.3.5 Antimicrobial Properties of green Tea (*Camellia sinensis*):

2.8.3.5.1 Microbiological effects:

There are different mechanisms for antimicrobial effects of green tea such as:

Polyphenols are anti-inflammatory agents that inhibit clinical symptoms of UTIs. Catechins induce production of cytokines such as IL-12 and IL-10. Green tea polyphenols decrease tumor necrosis factor- α gene expression, which is important in pathogenesis of *E. coli* infection. Catechins, by blocking the connection of conjugated R plasmid in *E.coli*, have bactericidal and antitoxin effects. Catechin-copper (II) complexes damage the cytoplasmic membrane of *E. coli*. EGC can bind to the ATP site of the DNA gyrase b subunit of bacteria and inhibit the activity of the gyrase enzyme. The bactericidal action of catechin is due to its hydrogen peroxide generation. The highest antimicrobial activity of tea is due to presence of catechins and polyphenols which damage the bacterial cell membrane. Catechins interfere with the expression of b-lactamases in *staphylococci* and inhibit the extracellular release of vero-toxin from *enterohemorrhagic E. coli* (EHEC) 0157 (Noormandi & Dabaghzadeh, 2015).

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. 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* (Hamilton-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. 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 patient. 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 (Hamilton-Miller, 1995).

2.8.3.5.2 Microbiological activities of green 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 polyphenols,

Sunphenon, prevented the attachment of a cariogenic *S. mutans* strain to hydroxy apatite and also inhibited its glucosyl transferase activity (Hamilton-Miller, 1995).

2.8.3.5.3 Microbiological activities of green 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 (Hamilton-Miller, 1995).

2.8.3.6 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. 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 & Zartoshti, 2007).

2.8.4 Previous studies:

Ikigai and his colleagues reported that EGCg and EC, two of the strongly antimicrobial catechins found in green tea. They used *E. coli* K-12 strain G6 and *Staphylococcus aureus* ATCC 25932 as Gram-negative and Gram-positive bacteria, respectively. EC and EGCg were extracted from water-soluble extract of green tea. The minimal growth inhibitory concentration (MIC) was determined by the agar dilution method. The MIC of EGCg for *E. coli* and *S. aureus* were 573 μg/mL and 73 μg/mL respectively. The MIC of EC for *E. coli* and *S. aureus* were >1145 μg/mL and 183 μg/mL, respectively. Catechins had greater activity against Gram-positive than Gram-negative bacteria. Liposomes were used as a model of bacterial membranes. EC showed little absorption through liposome membranes at 0.6 mM. They used EGCg to examine the effects of catechin on bacterial membranes. EGCg inhibited cytoplasmic membrane function by inducing leakage of small molecules from the intraliposomal space. Therefore,

catechins damaged bacterial membranes and impaired membrane function. (Ikigai, *et a.l*, 1993).

Reygaert and Jusifi in their study they used bacterial strains that were part of a research collection of *E. coli* isolated from UTI cultures during 2007–2008. Eighty isolates, which represent a wide spectrum of antimicrobial susceptibility patterns were selected from this collection; in addition, two control strains that were susceptible to all the clinically tested antimicrobials were selected. A standardized green tea (*C. sinensis*) extract (standardized to 7.0% polyphenols) was used. Luria–Bertani (LB) broth and dehydrated Müller–Hinton agar were used as media. Various concentrations of green tea extract (0 mg/mL, 2.5 mg/mL, 3 mg/mL, 3.5 mg/mL, and 4.0 mg/mL) were prepared and the MICs were determined by the agar dilution method. The results were as follows: 99% of strains were susceptible to the green tea extract at a concentration of ≤4.0 mg/mL (one strain was not susceptible at even 4.0 mg/mL); 94% of strains were susceptible at ≤3.5 mg/mL; 76% of strains were susceptible at ≤3.0 mg/mL; 40% of strains were susceptible at ≤2.5 mg/mL; and the control strains varied, one being susceptible at ≤2.5 mg/mL and the other susceptible at ≤3.5 mg/mL. Therefore, all of the strains tested, except one, had MICs of ≤4.0 mg/mL (99%). The results of this study show that green tea can have an antimicrobial effect on *E. coli* bacteria that causes UTIs. (Reygaert & Jusifi, 2013).

Kumar and his colleagues reported the antimicrobial activity of green tea extracts against various bacteria isolated from environmental sources. Different bacteria were isolated from sewage samples collected from different places at Solan

Himachal Pradesh. Isolated bacteria were identified by Gram staining and biochemical tests. A total of six different bacteria were identified (*Staphylococcus*, *Streptococcus*, *pseudomonas*, *E. coli*, *Proteus* and *Bacillus*). Aqueous, ethanolic, and air-dried and powdered extracts of green tea were prepared using standardized protocols. The disc diffusion method was used to test antimicrobial activity of all extracts, and antimicrobial assays were performed at concentrations of 10 μL, 20 μL, and 30 μL. For all extracts, significant antimicrobial activity was reported. Aqueous extract showed little antimicrobial activity against the six bacteria isolates; however, methanolic extract has shown maximum antibacterial activity (Kumar, *et al.,* 2012)

Vuong and his colleagues was studied *in vitro* experiments, but the effect can be described using information that is known about the metabolism of EGC from green tea. The amount of green tea polyphenols that would be present in the urine, including the amount of EGC, will vary according to the origin of the tea. It has been found, for instance, that Japanese tea (an average of 15 teas) contains approximately 20 mg of EGC per gram of dry tea. An average cup of Japanese green tea is made with one tablespoon of dry tea (instructions from package of tea) which is equivalent to approximately 7.5 g of dry tea (a package of 60 g of dry tea makes eight cups). That equates to approximately 150 mg of EGC per cup of Japanese green tea (Vuong, *et al.*, 2011).

Yang and his colleagues studied urinary excretion of ECG after ingestion of a single dose of it and found that the peak excretion to be after 8 h after ingestion,

with levels in the urine reaching to 3.0–5.0 mg. The amount of EGC excreted after ingestion of one cup of tea (as described above) should equal approximately 3.5 mg. Since the projected MICs for EGC are well below 3.5 mg, this suggests that even one cup of green tea could have an effect on a urinary tract pathogen, and drinking multiple cups over the course of a day could possibly provide a prolonged effect. Additional studies testing the *in vivo* effect of drinking green tea on UTIs could be useful for determining if the effects observed in this study have medical significance (Yang, *et al.*, 1998).

Cho and his colleagues claimed that concentrations of 500 μg of tea polyphenols can inhibit the growth of *E. coli*, and that concentrations of ≥5000 μg are considered bactericidal. This effect is believed to be due to the fact that tea polyphenols down regulate the production of proteins such as EF-2 (elongation factor for protein translation); proteins involved in phospholipid, carbon, and energy metabolism; and production of proteins involved in amino acid biosynthesis (Cho, *et al.*, 2007).

Other studies by Neyestani and his colleagues interesting and potentially useful would be to test EGC and several of the standard antimicrobial agents used to treat UTIs to determine if there might be synergism between EGC and any of the antimicrobial agents. Studies have been done that show the ability of green tea to act synergistically with gentamicin and amikacin against *E. coli* (Neyestani, et *al.*, 2007).

Radji and his colleagues studied the antimicrobial activity of green tea extract against isolates of methicillin-resistant *Staphylococcus aureus* and multi-drug resistant *Pseudomonas aeruginosa* and found that the MIC of green tea extract against MRSA was 400 µg/mL, while the MIC for MDR-*P. aeruginosa* was 800 µg/mL. The anti-bacterial activity of green tea extract was comparable to standard antibiotic. The activity of 16 µg green tea extract against laboratory strain of *S. aureus* ATCC 25923 was comparable to that of commercially available oxacillin (1 µg), whereas the activity of 16 µg green tea extract was comparable to that of commercially available gentamicin (10 µg) against laboratory strain of *P. aeruginosa* ATCC 27853, even though green tea extract was slightly less effective. Green tea extract showed good activity against MRSA and MDR-*P. aeruginosa*, although both of these bacteria showed resistance to multiple classes of antibiotics (Radji, *et al*., 2013).

A previous study by Lee his colleagues reported that green tea has anti-bacterial activity against resistant bacteria strains such as vancomycin-resistant enterococci, MRSA, and MDR-*P. aeruginosa*. Several previous studies have shown that green tea extract showed activity against both MRSA and methicillin-sensitive *Staphylococcus aureus* (Hamilton-Miller & Shah ,2000*)* and against MDR-*P. aeruginosa (*Lee, *et al.,* 2003). Evaluation of the antibacterial activity of water soluble green tea extracts on 43 hospital isolates of *Pseudomonas aeruginosa.* Antibacterial activity of water soluble green tea extract was measured by Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs). 35.6% of isolated strains showed resistance to 5

antibiotics or more and 55.8% of all strains were Multi-Drug Resistant (MDR) strains. The average MICs and MBCs of the extract against all strains of *Pseudomonas auroginosa* were 2.06 +/- 1.76 and 2.54 +/- 2.22 mg mL (-1) respectively.That study suggested that green tea has significant activity with bactericidal action on multi-drug resistant strains of *Pseudomonas aeruginosa* (Jazani, *et al*., 2007). Evaluation the antibacterial activity of water soluble green tea extracts on isolates of Acinetobacter was done. The susceptibilities of isolates to different antibiotics were tested using agar disk diffusion method. Antibacterial activity of water soluble green tea extract was measured by Minimum Bactericidal Concentrations (MBCs). Seventy five percent of isolated strains showed resistance to at least 12 antibiotics or more and all the strains were Multi-drug Resistant (MDR) strains. The average MBCs of the extract against all strains of *Acinetobacter* were 387.5 +/- 127.6 microg mL(-1).The Present study suggested that green tea has significant bactericidal action on multi-drug resistant strains of *Acinetobacter* (Jazani, *et al*., 2007).

Chapter 3

Materials and Methods

3. Materials and Methods

3.1 Methods:

3.1.1 Study design:

A cross-sectional, Hospital based study.

3.1.2 Study duration:

From February 2015 to February 2017.

3.1.3 Study population:

Pregnant females in Shendi hospitals.

3.2.3.1 Inclusion criteria:

Pregnant females in different ages and trimesters with urinary tract infection.

3.2.3.2 Exclusion criteria:

Specimens of Pregnant females negative for bacterial urinary tract infection and under treatment were excluded.

3.1.4 Sample size:

Sample was calculated from free online web site http://www.calculator.net by using confidence level 95% and confidence interval 8%, One hundred ninety one.

Sample size = $Z^2 \times (p) \times (p-1) / C^2$

Z = Z value – confidence level 95% (1.96),

C = Confidence interval (.08 = ± 8).

P = standard division (0.5).

The calculated sample size based on this formula was 191.

3.1.5 Scientific & Ethical considerations:

The study proposal was reviewed and ethically approved by the scientific and the ethical committee of Shendi University.

3.1.6 Data collection:

Data was collected by using questionnaire.

3.1.7 Study area:

Shendi locality, River Nile State, Sudan. Shendi is a town in northern of Sudan on the east bank of the River Nile 150 km northeast of Khartoum (16°41'N 33°25'E). The area is inhabited by the Ga'aleen Tribe.

3.1.8 Specimen collection:

Midstream urine (MSU) was collected as follows:

1. The patient was given a sterile, dry, wide-necked, leak proof container and requested to collect 10–20 ml of urine specimen.

2. The container was labeled with the date, the name and number of the patient, and the time of collection. When immediate delivery to the laboratory was not possible, the patient was requested to refrigerate the urine at 4–6 °C until delivery not more than 24 hours. (Cheesbrough, 2006).

3.1.9 Culture of urine specimen:

1. Urine sample were mixed well by rotating urine container several times.

2. Beside opened Bunsen burner urine container was opened and Nichrome loop was inserted after sterilization by flaming and cooling.

3. Small amount of urine sample was taken by loop and inoculated by making firstly well in Cystine lactose electrolyte deficient agar (CLED) media then

making primary lines from the well then secondary lines from primary lines then tertiary lines from secondary lines finally zigzag from last line of tertiary lines.

4. The inoculated plates were incubated in incubator at 37ºC for 24h under aerobic condition.

3.1.10 Interpretation of culture growth:

The plates were examined for any significant bacterial growth. The isolated bacteria were then identified by colonial morphology, Gram stain and biochemical tests.

3.1.11 Microscopic examination:

3.1.11.1 Preparation of smear:

1- On clean dry slide one drop of normal saline was putted and by loop after sterilization small amount of well grown single bacterial colony was taken from the agar plate and mixed with normal saline.

2- bacteria and normal saline were well mixed and spread on slide in area about 1 cm.

3- Slide was left to air dry then fixed by heating by flame by passing the slide in flame 3 times.

3.1.11.2 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).

Procedure:

1- After making heat fixed smear, the slide was putted in staining rack.

2- The smear was covered with the basic stain crystal violet then left for 1 minute.

3- Washed by tape water then covered the smear with the mordant lugol´s iodine for 1 minute then washed by tape water.

4- The smear was covered with the decolorizer 95% acetone alcohol for 5 seconds then washed by tape water.

5- Finally the smear was covered with the counter stain Saffranin and left it for 2 minutes then washed by tape water.

6- The smear was dried by air and examined under microscope using 100x lance.

Results:

Gram positive bacteria . . . . . Dark purple.

Gram negative bacteria . . . . . Pale to dark red. (Cheesbrough, 2006).

3.1.12 Biochemical tests:

3.1.12.1 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 a catalase producer. Hydrogen peroxide, 3% H_2O_2 (10 volume solution). 2–3 ml of the hydrogen peroxide solution was poured into a test tube. By using a sterile wooden stick or a glass rod (not a Nichrome wire loop), several colonies were removed of the test organism and immersed in the hydrogen peroxide solution. Immediately look for bubbling.

Result : (Appendix 5).

3.1.12.2 Coagulase test:

This test is used to identify *S. aureus* which produces the enzyme coagulase.

Principle:

Coagulase causes plasma to clot by converting fibrinogen to fibrin. EDTA anticoagulated human plasma (preferably pooled and previously HIV and hepatitis tested) or rabbit plasma. The plasma should be allowed to warm to room temperature before being used.

Procedure of Slide test method (detects bound coagulase):

1. One drop of distilled water was putted on each end of a slide or on two separate slides.

2. The colony was emulsified of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.

3. Loopful (not more) of plasma were added to one of the suspensions, and mixed

gently. Clumping of the organisms was looked within 10 seconds, No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Result : (Appendix 5).

3.1.12.3 DNA-ase test:

Principle:

Deoxyribonuclease hydrolyzes deoxyribonucleic acid (DNA).The test organism is cultured on a medium which contains DNA. After overnight incubation, the colonies are tested for DNA-ase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. DNA-ase-producing colonies are therefore surrounded by clear areas due to DNA hydrolysis. DNA-ase agar plate Up to six organisms may be tested on the same plate. Hydrochloric acid 1 mol/1 (1N).

Result : (Appendix 5).

3.1.12.4 Mannitol Salt Agar (MSA):

This type of medium is both selective and differential. The MSA will select for organisms such as *Staphylococcus* species which can live in areas of high salt concentration.

Result : (Appendix 5).

3.1.12.5 Oxidase test (Cytochrome oxidase test):

The oxidase test is used to assist in the identification of *Pseudomonas*, *Neisseria*, *Vibrio*, *Brucella*, and *Pasteurella* species, all of which produce the enzyme cytochrome oxidase.

Method using an oxidase reagent disc:

1. One disc was putted of oxidase disc on flat surface.

2. By using a piece of stick or glass rod (not an oxidized wire loop) a colony of the test organism was removed and rubbed on the disc.

3. A purple color was looked within 10 seconds.

Result : (Appendix 5).

3.1.12.6 Urease test:

Testing for urease enzyme activity is important in differentiating enterobacteria.

Principle:

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in color of the indicator to pink-red.

3.1.12.7 Indole test:

Principle:

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethyl aminobenzaldehyde.This reacts with the indole to produce a red colored compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

Detecting indole using peptone water:

1. The test organism was inoculated in a tube containing 3 ml of sterile peptone

water.

2. Then Incubated at 37ºC for 24 h.

3. Indole was tested by adding 0.5 ml of Kovac'sreagent. Shaked gently. A red color in the surface layer within 10 minutes were examined.

Result : (Appendix 5).

3.1.12.8 Citrate utilization test:

This test is one of several techniques used occasionally to assist in the identification of enterobacteria.The test is based on the ability of an organism to use citrate as its only source of carbon.

Citrate method using Simmon's citrate agar:

1. Slopes of the medium were prepared in bijou bottles as recommended by the manufacturer.

2. Using a sterile straight wire, firstly the slope was streaked with the test organism and then stab the butt.

3. At 35ºC for 24 hours media was incubated. Then looked for a bright blue color in the medium.

Result : (Appendix 5).

3.1.12.9 Kliger's Iron Agar (KIA):

This is a differential medium. It tests for organisms' abilities to ferment glucose and lactose to acid and acid plus gas end products. It also allows for identification of sulfur reducers. This media is commonly used to separate lactose fermenting members of the family *Enterobacteriaceae* (e.g. *Escherichia coli*) from members that do not ferment lactose.

Principle:

The first differential ingredient, glucose, is in very short supply. Organisms capable of fermenting this sugar will use it up within the first few hours of incubation. Glucose fermentation will create acidic byproducts that will turn the phenol red indicator in the media yellow. Thus, after the first few hours of incubation, the tube will be entirely yellow. At this point, when the glucose has been all used up, the organism must choose another food source. If the organism can ferment lactose, this is the sugar it will choose. Lactose fermentation will continue to produce acidic byproducts and the media will remain yellow (picture on the far left below). If gas is produced as a result of glucose or lactose fermentation, then fissures will appear in the agar or the agar will be lifted off the bottom of the tube. If an organism cannot use lactose as a food source it will be forced to use the amino acids / proteins in the media. The deamination of the amino acids creates NH_3, a weak base, which causes the medium to become alkaline. The alkaline pH causes the phenol red indicator to begin to turn red. Since the incubation time is short (18-24 h), only the slant has a chance to turn red and not the entire tube. Thus an organism that can ferment glucose but not lactose will produce a red slant and a yellow butt in a KIA tube (second from the left below). These organisms are the more serious pathogens of the GIT such as *Shigella dysenteriae* (MacFaddin, 1980).

Result : (Appendix 5).

Procedure:

1. The KIA agar slants were labeled with the name of the bacterium to be

inoculated. One of the tubes was used as a control.

2. Aseptic technique was used, the slant was streaked with the appropriate bacterium and then the butt was stabbed. The caps on the tubes were screwed but do not tighten!

3. Only for 18 to 24 hours at 35°C media was incubated for changes in the butt and on the slant. Tubes should be incubated and checked daily for up to seven days in order to observe blackening (John, 2002).

Result : (Appendix 5).

3.1.12.10 Litmus milk decolorization test:

This test is a rapid in expensive technique to assist in the identification of *Enterococci*. It is based on the ability of most strains of *Enterococcus* species to reduce litmus milk by enzyme action as shown by decolorization of the litmus.

Method:

1. Sterile loop was used; 0.5 ml of sterile litmus milk medium was inoculated with the test organism.

2. At 37ºC for up to 4 hours media was incubated, at half hour intervals media was examined for a reduction reaction as shown by a change in color from mauve to white or pale yellow (compared with the positive control).

Result : (Appendix 5).

3.1.12.11 Bile Esculin Agar slant:

This is a medium that is both selective and differential. It tests the ability of organisms to hydrolyze esculin in the presence of bile. It is commonly used to identify members of the genus *Enterococcus.*

Principle:

Bacteria hydrolyze esculin to produce esculitin and glucose, Esculitin reacts with ferric chloride to form black precipitate in media.

Procedure:

1. Slopes of the medium were prepared in bijou bottles as recommended by the manufacturer.

2. Sterile straight wire was used, firstly the slope was streaked with the test organism and then stab the butt.

3. At 35ºC for 24 hours media was incubated.

Result : (Appendix 5).

3.1.13 Preparation of plant extract:

The plant extracts were prepared using the solvent water. 15g of green tea dry leaves (Veitnam green tea) minced to powder were taken and homogenized with100 ml of the distil water (Kumar, *et al.*, 2012). The mixture was left in hot air oven under 80ºC for 2 hours (Uzunalic, *et al.*, 2006). After that left to cool and filter the mixture by piece of gauze then by Whatman filter paper No.1. The extract was heated in hot air oven under 60ºC to make stock crude material. The crude was weight by sensitive balance and 0.5g then dissolved in 1ml of sterile distil water to obtain concentration 500mg/ml then diluted in tubes by using two fold dilution to make concentration 250.125,62 and 31 mg/ml.

3.1.14 Procedure of inoculation in Mueller Hinton agar plates and applying green tea extract:

1- By the loop the tops of each of 3–5 colonies were touched, of similar

appearance, of the organism to be tested.

2- The growth was transferred to a tube of sterile saline and mixed then compared the tube with the turbidity standard and adjusted the density of the test suspension to that of the standard by adding more bacteria or more sterile saline.

3- The plates were inoculated by dipping a sterile swab into the 37noculums. The excess 37noculums was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid.

4- The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60º after each application. Finally, the swab was passed round the edge of the agar surface. The inoculums was left to dry for a few minutes at room temperature with the lid closed.

5- By using glass porer of size 6 mm in diameter, 5 pores were made in agar plate then the pores were filled 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- The plates were incubated for 24h in incubator under aerobic condition in 37ºC.

3.1.15 interpreting the sensitivity of green tea extract:

1- The diameter of each zone (including the diameter of the disc) had been measured and recorded in mm.

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

3.1.16 Calculation of relative percentage of inhibition:

Relative percentage inhibition = 100 x (x – y) / (z – y)

x: total area of inhibition of the test extract.

y: total area of inhibition of the solvent.

z: total area of inhibition of the standard drug.

The total area of the inhibition was calculated by using area = πr^2; where, r = radius of zone of inhibition.

- π value = 3.14

X: Total area of inhibition of the test extract = 3.14 x (radius of zone inhibition of green tea extract in mm)^2.

Y: Total area of inhibition of the solvent = 3.14 x (radius of zone inhibition of water in mm)^2.

Z: Total area of inhibition of the standard drug = 3.14 x (radius of zone inhibition of Gentamicin in mm)^2.

3.1.17 *Statistical analysis:*

Data was analyzed by using online web site https//www.graphpad.com. Proportional data were presented as frequencies and percentages.

3.2 Materials

3.2.1 Mueller–Hinton agar:

Mueller–Hinton agar was prepared from a dehydrated base according to the manufacturer's instructions.

3.2.2 Green tea leaves:

Dried Vietnam green tea leaves.

3.2.3 Turbidity standard (0.5 McFarland standard):

The turbidity standard was prepared by pouring 0.6 ml of a 1% (10 g/l) solution of barium chloride dihydrate into a 100 ml graduated cylinder, and filling to100ml with 1% (10 ml/l) sulfuric acid. The turbidity standard solution was 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 (Vandepitte, *et al*. 2003).

3.2.4 Swabs:

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

3.2.5 Sterile Normal saline concentration 0.85%:

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

3.2.6 Disposable plastic Petri dish:

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

3.2.7 Glass porer:

6 mm diameter glass porer.

3.2.8 Automatic pipette:

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

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

3.2.9 Disposable plastic automatic pipette tips:

- Blue tips (size: 1000 µ.l)

- Yellow tips (size: 200 µ.l).

3.2.10 Serology small glass tubes:

12 x 75mm glass test tube.

3.2.11 large size glass tubes:

15 x100mm glass test tubes.

3.2.12 glass Erlenmeyer flask:

500ml size flask and 250ml size flask.

3.2.13 glass Beaker:

300 ml size.

3.2.14 bacteriological loops:

- Nichrome ring loop and needle loop (HI-MEDIA).

3.2.15 test tubes racks.

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

Chapter 4

Results

4. Results:

A total of one hundred ninety one pregnant women with UTI patients were enrolled in this study. The mean age was (25.4 ± 6.7) years. Most of them were within the age group less than 24 and 25 – 34 years (47.7%, 47.1%) respectively (Table 1).More than 50% were in their 3d trimester (Table 2).

4.1 Tea drinking during pregnancy:

One hundred forty six (76%) of the pregnant women in this study were drinking tea during pregnancy and 145 (99.3%) drink black tea showed in (Table 3).

4.2 Isolated and identified bacterial pathogens:

The main causative agent of UTI in the study population was *S. aureus* (24.8%), followed by *E. coli* (21.9%) and the least causative agents were *Hafnia alvei* and *Proteus vulgaris* (0.7 %) (Table 4). The Gram negative bacilli isolated were *E. coli* represented the main causative agent between Gram negative bacilli bacteria 30 (50) and lowest causative Gram negative bacilli were *Proteus vulgaris* and *Hafnia alvei* 1 (1.6%) (Table 5). The Gram positive cocci isolated was *S. aureus* 34 (44.2%) and it is the main causative agent between Gram positive cocci bacteria and lowest causative Gram positive cocci was *Enterococcus fecalis* 5 (6.5 %) (Table 6).

4.3 Pregnancy trimester and urinary bacterial pathogens:

In 1st trimester *Citrobacter freundii* was the main causative agent of UTI 5 (29.4%), *E. coli* and *Klebsiella pneumonae* were least isolated agents 1 (5.9%).

In 2nd trimester *S. aureus* was the main causative agent of UTI 10 (27.1%), *Enterobacter cloacae*, *S. saprophyticus* and *E. fecalis* were least isolated agents 1 (2.7%).

In 3d trimester also *S. aureus* was the causative agent of UTI 21 (25.3%), *Proteus vulgaris, Hafnia alvaei, Citrobacter freundii* and *Enterobacter cloacae* were least isolated agents1 (1.2%) (Table 7).

4.4 Susceptibility testing of the green tea:

. Antimicrobial susceptibility of green tea extract has largest zone of inhibition against *Enterococcus fecalis* was (17.6 ± 1.9 mm) and least zone of inhibition against *Proteus vulgaris* and *Enterobacter cloacae* was (8 ± 0 mm) (Table 8).The results of antimicrobial activity of crude extract was compared with the positive control (Standard drugs) for evaluating their relative percentage inhibition, the aqueous extract exhibited maximum relative percentage inhibition against *S. aureus* (38.2%) and minimum relative percentage inhibition against *Enterobacter cloacae* was (11.1%) (Table 9).

4.5 Minimum inhibitory concentration*:*

Antibacterial activities of extracts were checked by well diffusion method. The concentrations of green leaves aqueous, extract used was 500, 250, 125, 62.5 and 31 mg/ml.MIC values of aqueous extracts of green tea on test organisms which the lowest concentration of green tea aqueous extract able to inhibit the growth of

bacteria was (48.6 mg/ml) appear against *S. aureus* followed by *Staphylococcus saprophyticus* (58 mg/ml), *Enterococcus fecalis* (62.8 mg/ml), *Micrococcus luteus* (66.8 mg/ml) *Staphylococcus epidermidis* (68.2 mg/ml), *Hafnia alvei* (250 mg/ml), *E. coli* (280.2 mg/ml), *Citrobacter freundii* (312.5 mg/ml), *Citrobacter diversus* (325 mg/ml), *Enterobacter cloacae*. *Klebsiella pneumonae* and *Proteus vulgaris* (500mg/ml) (Figure 1).

Table 1: Show distribution of the population according to age.

Age group	Number	%
Less than 24	91	47.7%
25 – 34	90	47.1%
35 – 44	9	4.7%
More than 44	1	0.5%
Total	**191**	**100%**

Age (mean ± SD) = (25.4 ± 6.7)

Table 2: Show distribution of the population according to trimester.

Trimester	Number	%
First	24	12.6%
Second	60	31.4%
Third	107	56.0%
Total	**191**	**100**

Table 3: Show distribution of population according to tea type used.

Type of tea used	Number	%
Black	145	99.3%
Green	1	0.7%
Total	**146**	**100%**

Table 4: Show percentage of bacteria isolated from urine of study population

Bacteria name	Number	%
1. *Citrobacter diversus*	10	7.30%
2. *Citrobacter freundii*	6	4.40%
3. *Escherchia coli*	30	21.9%
4. *Enterobacter cloacae*.	2	1.50%
5. *Enterococcis fecalis*	5	3.60%
6. *Hafnia alvei*	1	0.70%
7. *Klebsiella pneumonae*	10	7.30%
8. *Micrococcus luteus*	15	11%
9. *Proteus vulgaris*.	1	0.70%
10. *Staphylococcus aureus*	34	24.8%
11. *Staphylococcus epidermidis*	16	11.70%
12. *Staphylococcus saprophyticus*	7	5.10%
Total	**137**	**100**

Table 5: Show Gram negative Bacteria isolated from urine of study population:

Bacteria isolated	Number	%
1. *Citrobacter diversus*	10	16.7%
2. *Citrobacter freundii*	6	10%
3. *Escherchia coli*	30	50%
4. *Enterobacter cloacae.*	2	3.4%
5. Hafnia alvei	1	1.6%
6. Klebsiella pneumonia	10	16.7%
7. Proteus vulgaris	1	1.6%
Total	**60**	**100%**

Table 6: Show Gram positive Bacteria isolated from urine of study population:

Bacteria isolated	Number	*%*
1. *Staphylococcus aureus*	34	44.2%
2. *Staphylococcus epidermidis*	16	20.7%
3. *Staphylococcus saprophyticus*	7	9.1%
4. Enterococcus fecalis	5	6.5%
5. Micrococcus luteus	15	19.5%
Total	**70**	**100%**

Table 7: Show percentage of isolated bacteria from urine of pregnant woman according to trimester

Bacteria name	Total No.	1st trimester		2nd trimester		3d trimester	
		n	%	n	%	n	%
Citrobacter diversus	10	0	0%	4	10.8%	6	7.2%
Citrobacter freundii	6	5	29.4%	0	0%	1	1.2%
Escherchia coli	30	1	5.9%	9	24.3%	20	24.1%
Enterobacter cloacae.	2	0	0%	1	2.7%	1	1.2%
Enterococcis fecalis	5	4	23.6%	1	2.7%	0	0%
Hafnia alvei	1	0	0%	0	0%	1	1.2%
Klebsiella pneumonae	10	1	5.9%	3	8.1%	6	7.2%
Micrococcus luteus	15	3	17.6%	5	13.5%	7	8.5%
Proteus vulgaris.	1	0	0%	0	0%	1	1.2%
Staphylococcus aureus	34	3	17.6%	10	27.1%	21	25.3%
Staphylococcus epidermidis	16	0	0%	3	8.1%	13	15.7%
Staphylococcus saprophyticus	7	0	0%	1	2.7%	6	7.2%
Total	**137**	**17**	**100%**	**37**	**100%**	**101**	**100%**

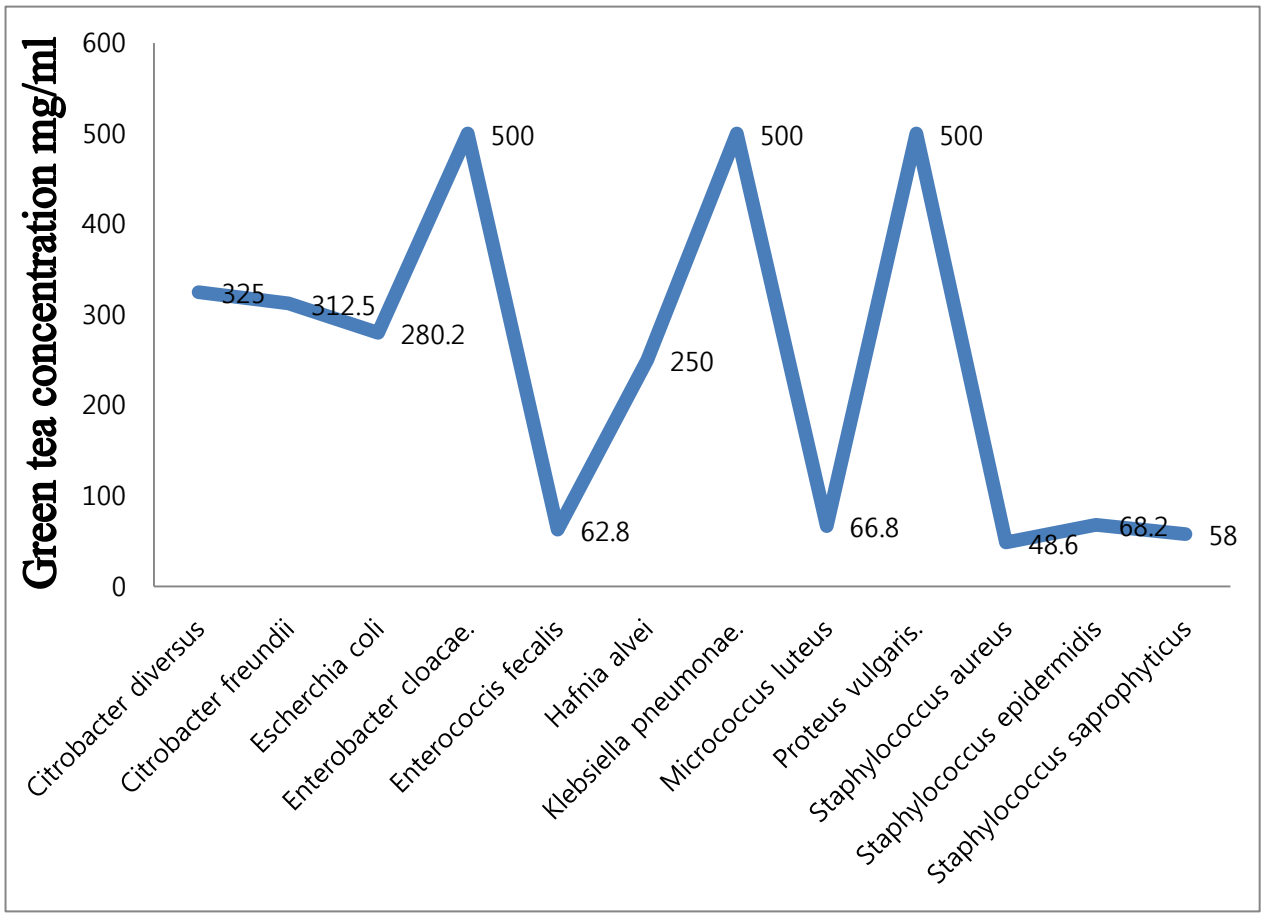

Figure 1: MIC values of aqueous extracts of green tea on test organisms (mg/ml)

Table 8: Show antimicrobial susceptibility of green tea extract compared to Gentamicin.

Bacteria isolated	Inhibition zone diameter (mm)	
	Aqueous extract (Green tea 500 mg/ml)	**Positive control (Gentamicin 10 mcg)**
1- Citrobacter diversus.	9.1 ± 0.9	19.5
2- Citrobacter freundii	9.1 ± 2.1	19
3- Escherchia coli.	10.8 ± 3.0	18
4- Enterobacter cloacae.	8 ± 0	24
5- Enterococcis fecalis.	17.6 ± 1.9	29
6- Hafnia alvei.	9 ± 0	20
7- Klebsiella pneumoniae.	8.6 ± 2.5	23
8- Micrococcus luteus.	16.5 ± 1.9	28.5
9- Proteus vulgaris.	8 ± 0	20
10- Staphylococcus aureus.	18.0 ± 2.4	29.1
11- Staphylococcus epidermidis.	16.8 ± 2.1	28.9
12- Staphylococcus saprophyticus.	16.8 ± 2.5	30

Table 9: Show the relative percentage inhibitions of green tea extract compared to Gentamicin.

Test organisms	Relative percentage inhibition (%)
1- Citrobacter diversus.	21.8%
2- Citrobacter freundii	21.8%
3- Escherchia coli.	36%
4- Enterobacter cloacae.	11.1%
5- Enterococcus fecalis.	36.8%
6- Hafnia alvei.	20.2%
7- Klebsiella pneumoniae.	13.9%
8- Micrococcus luteus.	33.5%
9- Proteus vulgaris.	16%
10- Staphylococcus aureus.	38.2%
11- Staphylococcus epidermidis.	33.7%
12- Staphylococcus saprophyticus.	31.3%

Chapter 5

Discussion

Conclusion

Recommendations

5.1 Discussion:

Urinary tract infection is a common health problem among pregnant women (Alemu, *et al*., 2012). The prevalence of UTI among pregnant women in Sudan was 14.0% (Hamdan, *et al.,* 2011). This usually begins in week six and peaks during weeks twenty two to twenty four of pregnancy due to a number of factors including urethral dilatation, increased bladder volume and decreased bladder tone, along with decreased urethral tone which contributes to increased urinary stasis and ureterovesical reflux and up to 70% of pregnant women develop glycosuria, which encourages bacterial growth in the urine (Van Brummen, *et al*., 2006). The main causative agent of UTI in the study population was *S. aureus* (24.8%), it has been reported to be the most frequent pathogen among pregnant women, this is in agreement with report from Nigeria **(**Akinloye, *et al.,* 2006), followed by *E. coli* (21.9%). The green tea extract had an inhibitory effect on the growth of *E. coli* strains isolated from UTIs **(**Reygaert & Jusufi, 2013).

. In current study antimicrobial susceptibility of aqueous green tea extract show highest zone of inhibition against *Enterococcus fecalis* (17.6 ± 1.9 mm) and lowest zone of inhibition against *Proteus vulgaris* and *Enterobacter cloacae* (8 ± 0 mm).The highest antimicrobial activity of tea is due to presence of catechins and polyphones which damages bacterial cell membrane (Kumar, *et al.,* 2012). **Ikigai** and colleagues reported that tea catechins have less effect on Gram negative bacterial cell membrane due to the fact that LPS outer membrane of Gram negative is negatively charged (IKigai, *et al*., 1993), Kumar and

colleagues reported that the daily consumption of green tea can kill Gram positive *Staphylococcus aureus* including many other harmful bacteria(Kumar, *et al.,* 2012), **Alizadeh** and Mohebalian reported that the maximum zone of inhibition was observed against *S. aureus* (A Gram positive organism) and the minimum was against pseudomonas (Alizadeh & Mohebalian, 2016). Studies have shown that concentrations of 500 μg of tea polyphenols can inhibit the growth *of E. coli*, and that concentrations of ≥5000μg are considered bactericidal. This effect is believed to be due to the fact that tea polyphenols down regulate the production of proteins such as EF-2 (elongation factor for protein translation); proteins involved in phospholipid, carbon, and energy metabolism; and production of proteins involved in amino acid biosynthesis **(**Reygaert & Jusufi, 2013).

In scientific research, a growing trend is observed toward studies analyzing functional food, in addition to food source, part of them or isolated substances have had their pharmacological activity demonstrated as capable of promoting health benefits, in prevention and/ or treatment of diseases (Enzveiler, *et al.*, 2011). Antimicrobial property in tea is due to presence of polyphenols. Specific antioxidant polyphenols (Kumar, *et al.*, 2012). Some of the polyphenols may confer their beneficial effects to specific parts of the body, in which they are concentrated. This explanation is further complicated by the immune system interactions with both polyphenolic compounds and the invasive pathogen (Neyestani, *et al.,* 2007). Polyphenols called catechins, play an important role in green tea's inhibition of bacterial growth. Several significant

catechins include: epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epicatechin (EC), and gallocatechin-3-gallate (GCG) (Kumar, *et al.,* 2012)

MIC values of aqueous extracts of green tea on test organisms which the lowest concentration of green tea aqueous extract able to inhibit the growth of bacteria is (48.6 mg/ml) appear against *S. aureus* followed by *Staphylococcus saprophyticus* (58 mg/ml), *Enterococcis fecalis* (62.8 mg/ml), *Micrococcus luteus* (66.8 mg/ml) *Staphylococcus epidermidis* (68.2 mg/ml), *Hafnia alvei* (250 mg/ml), *Escherchia coli* (280.2 mg/ml), *Citrobacter freundii* (312.5 mg/ml), *Citrobacter diversus* (325 mg/ml), *Enterobacter cloacae. Klebsiella pneumonae and Proteus vulgaris* (500mg/ml). Our finding is in disagreement with **Hoseeni** and his colleagues who reported that effect of water soluble extracts of green tea on the activity of Ciprofloxacin in urinary isolated *E. coli* that the MIC of aqueous green tea extract against *E. coli* was (122.9 mg/ml), (Hoseeni & Zartoshti,2007). **Neyestani** and colleagues investigated microbiologic effects of tea extract on certain antibiotics against *E. coli* in vitro. They used bacterial strain ATCC 25920 and crude tea extracts. Different concentrations of black or green tea extracts (6.25 mg/mL, 12.5 mg/mL, 25 mg/mL, 50 mg/mL, and 100 mg/mL) were used for this study. They used the method of disc diffusion for bacterial sensitivity tests. Green tea at 20 mg/mL concentration inhibited *E. coli* growths completely (Neyestani, et al., 2007) which disagree with the study results. We suggested that green tea that prepared with liquid chromatography show high performance than prepared with dry

oven.

The results of antimicrobial activity of crude extract was compared with the positive control (Standard drugs) for evaluating their relative percentage inhibition while the aqueous extract exhibits maximum relative percentage inhibition against *S. aureus* (38.2%) and minimum relative percentage inhibition against *Enterobacter cloacae* (11.1%).

5.2Conclusion

The green tea extract has ability to inhibit the growth of most bacteria that cause urinary tract infection.

The lowest concentration of green tea aqueous extract able to inhibit growth of bacteria that cause UTI is 48.6 mg/ml.

The inhibitory effect of green tea aqueous extract is better in Gram positive bacteria than Gram negative according to MIC and zone of inhibition results.

5.3 Recommendations

Making more studies about effect of aqueous green tea extract on bacterial infection happens in other body systems.

Data from in vitro studies on the antimicrobial effects of green tea are promising, but human data are currently lacking. Therefore, it is essential to have in vivo studies on antibacterial effects of green tea.

Human clinical trials also need to evaluate the synergistic effect between green tea and antibiotics used in UTIs and evaluated the efficacy of its catechins in the treatment of UTIs in the future.

Chapter 6

References

Appendix

6.1References:

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6.2 Appendices

Appendix 1: Questionnaire

University of Shendi

Faculty of medical laboratory sciences

College of Graduate Studies

In Vitro Susceptibility Patterns Of Aqueous Extract Of Green Tea On Bacteria Isolated From Pregnant Women With Urinary Tract Infection.

Name:……

State:……

City:……

Village:……

code: ☐

1- **Age:**

a- ≤ 24 (……) c- 25 – 34 (……). d- 35 – 44 (……...). e- > 44 (……).

2- **Occupations:**

a- House wife (……) b- Other (……)

3- **Pregnancy trimester:**

a- First trimester (……). b- Second trimester (……). c- Third trimester (…….).

4- **Treatment:**

a- Treated with antibiotic (……). b- Not treated (……).

5- **If under treatment, the name of treatment used**……

6- Response to treatment:

a- Good (…..) b- Few (……) c- No response (……)

7- Drinking tea during pregnancy:

a- Never (……). b- Once a day (…….).

c- Twice a day (……). d- Three times a day (……).

8- If you drinking tea, which type you drink?

a- Green tea (……). b- Black tea (……).

9- History of urinary tract infection in previous pregnancy:

a- Yes (……) b- No (……)

10- Other disease:

a- Diabetes (…….) b- Hypertension (……) c- Other (……) d- No (……)

Appendix 2: Plates

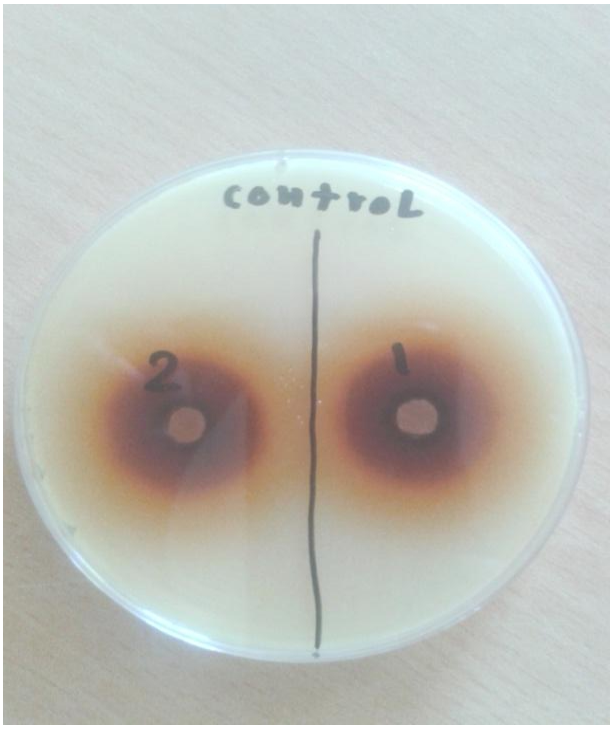
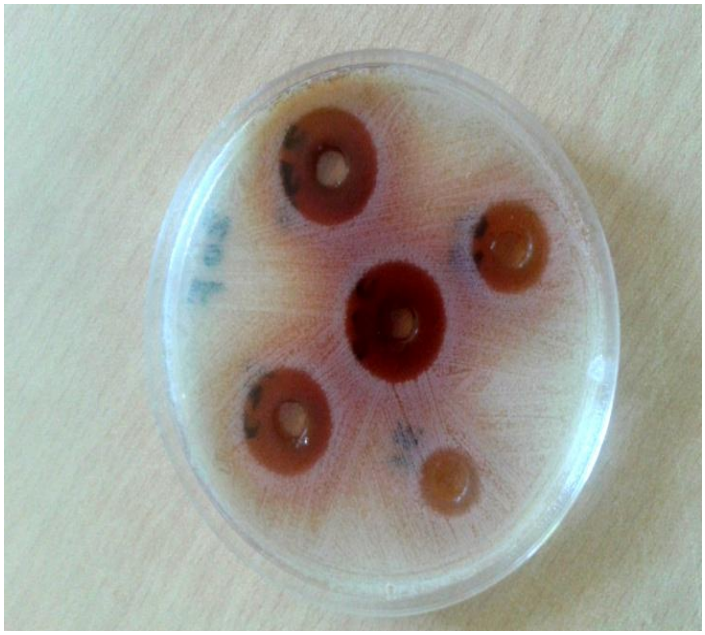


Plate 1: Green tea extract inhibition zone in control strain *S.aureus* ATCC



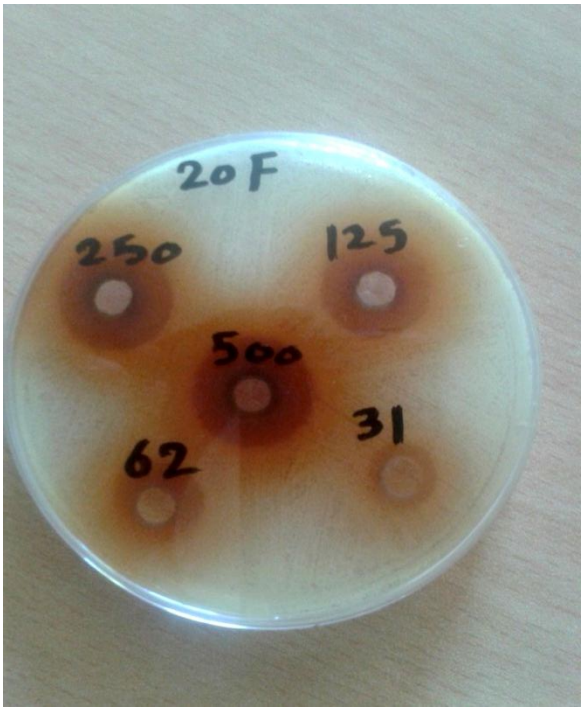


Plate 2: Green tea extract inhibition zones at different concentrations (500, 250, 125, 62 and 31 mg/ml)

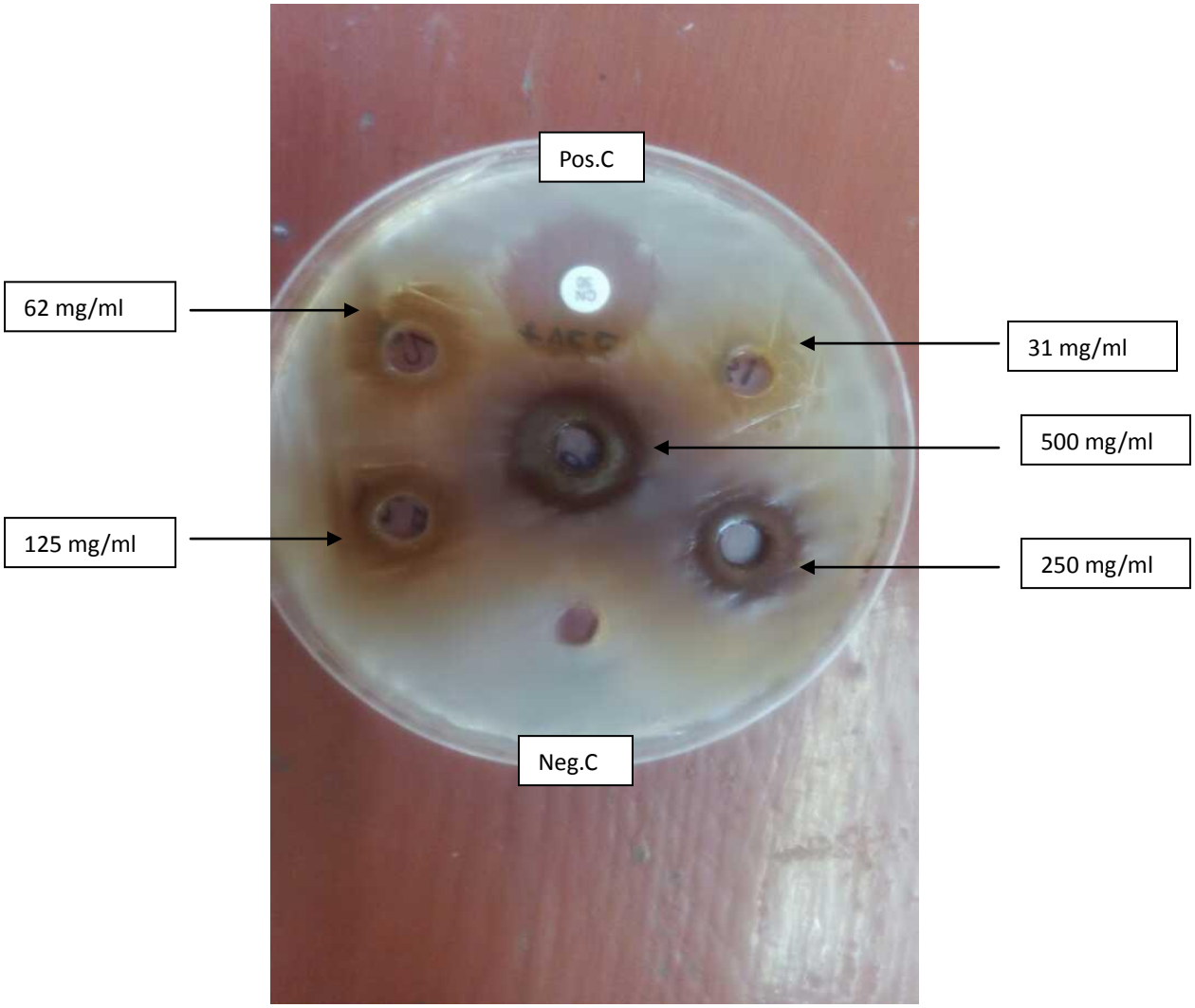


Plate 3: Green tea extract inhibition zones at different concentrations (500, 250, 125, 62 and 31 mg/ml) with positive control (Gentamicin) and negative control (Distil water)

Appendix 3: 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 enzymic hydrolysate. -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. -Sodium chloride. -Bromothymol blue. -Agar.	0.2 1.0 1.0 2.0 5.0 0.08 15.0	Suspend 24.28 grams in 1000 ml distilled water. Heat, to boiling, to dissolve 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

Appendix 4: Biochemical tests reactions of isolated bacteria.

Biochemical reactions of isolated Gram negative bacilli

Bacteria name	Oxidase test	Urease test	Motility test	Citrate utilization test	Indol test	KIA			
						H_2S	Gas	Glucose	Lactose
E.coli	-	-	+	-	+	-	+	+	+
C.diversus	-	-	+	+	+	-	+	+	+
C.freundii	-	+	+	+	-	-	+	+	-
Klebsiella pnumoniae	-	+	-	+	-	-	+	+	+
P.vulgaris	-	+	+	+	+	+	+	+	-
E.cloacae	-	-	+	+	-	-	+	+	+
H.alvei	-	-	-	-	-	-	-	+	-

Biochemical reactions of isolated Gram positive cocci

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

Biochemical reactions of isolated Gram positive cocci

Bactria name	Catalase test	Litmus milk decolorization test	Esculin hydrolysis test	Arabinose sugar fermentation	Salt tolerance test in 6.5% Nacl	Heat resistant test
Enterococcus fecalis	-	+	+	-	+	+