بسم الله الرحمن الرحيم

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Determination of Invitro Antibacterial Activity of Ginger Extract on Bacteria Isolated from Diabetic Patient with Urinary Tract Infections in Shendi Locality

A thesis Submitted for partial fulfillment of the Msc Degree in Microbiology

By

Ibtihal Ibrahem Mohammed Ahmed

Bsc. (Shendi University – 2011 Microbiology –Clinical Chemistry)

Supervisor

Dr: Leila Mohammed Ahmed

PhD in Microbiology

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الآيـــــة

بسم الله الرحمن الرحيم

قال تعالى:

﴿ وَيُسْقَوْنَ فِيهَا كَأْساً كَانَ مِزَاجُهَا زَنجَبِيلاً ﴾

صدق الله العظيم

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Dedication

To my wonderful parents who strongly supported me all throughout.

To my beloved sisters and adorable brothers.

To my lovely family.

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

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First of all I would like thank Allah, for giving me health strength and patience to complete this work. I would like to formally thank:

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List of abbreviations

Abbreviation	Mean
CT	Computed Tomography
DM	Diabetes Mellitus
DV	Daily Value
E.coli	Escherium Coli
EC	Emphysematous Cystitis
E.fecalis	Enterococus Fecalis
EP	Emphysematous Pyelitis
EPN	Emphysematous Pyelonephritis
IVU	Intravenous Urography.
MRI	Magnetic Resonance Imaging
RPN	Renal Papillary Necrosis
S.aureus	Staphylococus Aureus
S.saprophyticus	Streptococus Saprophyticus
US	Ultra Sound
UTIs	Urinary Tract Infections
WHO	World Health Organization
XGP	Xanthoranulomatous Pylonephritis

Abstract

Background: Urinary tract infections frequently occur in diabetic patients due to an impaired immune status and increased glucose content of the urine. among other reasons. This make UTI very important to investigate. Complicated cases of UTI maybe in frequent but are more common in diabetics with for more severe consequences. And so warrant further investigations. The proper management of UTI in diabetics is crucial as prompt diagnosis and correct use of antibiotic is vital for treatment. Finding alternative antimicrobial agents from plant extracts has received growing interest. *Ginger* (*Zingiber officinale*) a safe, non toxic, cheap spice that has been reported to have antimicrobial effects against various pathogenic bacteria..

Objectives: This study aimed to evaluate the effectiveness of ginger extract on different types of bacteria isolated from diabetic patient suffering from urinary tract infection.

Methodology: A cross-sectional and hospital based study, has been conducted in University of Shendi –faculty of medical laboratory sciences- department of microbiology from May to November 2018. Following informed consent 100 diabetic patient suffering from UTI in different ages were enrolled in this study. sixty two bacteria were isolated, different Gram positive and Gram negative bacteria, in vitro sensitivity testing using well diffusion technique against ginger extract.

Results: The main causative agent of UTI in the study population was *E. coli* 32.3% then *S. aureus* 29%, *S.saprophyticus* 29% and *E.fecales* 9.7%. The largest diameter of inhibition zone appeared in Gram positive *S.saprophyticus* (13.4 mm). The concentrations of ginger extract used was 100, 50, 25 and 12.5 mg/ml. mean of inhibition zone of chloramphicol for isolated bacteria *S.aureus* 31mm, *S.saprophyticus* 20,2 mm, *E.coli* 17.7mm and *E.fecalis* 24.6mm.

Conclusion: The antimicrobial activity of crude extract was compared with that of standard antimicrobial chloramphnicol based on the mean diameter of inhibition zone. The extract exhibited maximum relative percentage inhibition against *S.saprophyticus* (42.3%) and minimum relative percentage inhibition agains t *S.aureus* was (6.3%).

المستخلص

المقدمة: تحدث التهابات المسالك البولية في كثير من الأحيان عند مرضى السكري نتيجة لضعف المناعة وزيادة محتوى السكر في البول وأسباب أخرى مما يجعل التهاب المسالك البولية مهم التشخيص والحالات المعقدة نادرة الحدوث ولكن أكثر شيوعا في مرضى السكري التهاب المسالك البولية يحتاج للتشخيص الفوري والعلاج السليم.وجود بدائل من عوامل مضادة للميكروبات مستخلصة من النبات وجد طريقا للاهتمام. نبتة القنقر (الزنجبيل)هي نبتة آمنة، غير سامة و رخيصة الثمن للتحصل عليها و قد أثبتت التقارير أن لها فعالية مضادة للميكروبات الممرضة.

الهدف :هدفت هذه الدراسة إلى معرفة مدى تأثير مستخلص لنبتة القنقر (الزنجبيل) على الأنواع المختلفة للبكتيريا المعزولة من مرضى السكري يعانون من إخماج المجاري البولية.

المنهجية : أجريت هذه الدراسة التقاطعية في جامعة شندي– كلية علوم المختبرات الطبية – قسم الأحياء المجهرية في الفترة من مايو إلى نوفمبر ٢٠١٨م . وقد تضمنت عدد ١٠٠ عينة من مرضى السكر يعانون من إخماجات المجاري البولية من أعمار مختلفة. اثنين وستون بكتيريا معزولة مختلفة الأنواع موجبة وسالبة الجرام مسببة لإخماج المجاري البولية أختبر تأثير المستخلص الزنجبيل عليها داخل المعمل باستخدام تقنية الثقوب النافذة أو المنتشرة .

النتائج:

العامل الرئيسي المسبب لاخماج المسالك البولية في مجتمع الدراسة هو الاشريكية القولونية(٠٣٢,٣%) تليها المكورات العنقودية الذهبية والمكورات العنقودية المترممة (٢٩%) واخيرا المكورة العنقودية البرازية (٩,٧%).اكبر نطاق للتثبيط ظهر ضد البكتيريا موجبة الجرام المكورة العنقودية المترممة (١٣,٤مم).التراكيز المستخدمة لمستخلص الزنجبيل هي ١٠٠،٥٠،٢٥،١٢,٥جرام /مل .معدل التثبيط للمضاد الحيوي للكلورافلنيكول المكورة العنقودية الذهبية (٣١مم) والمكورات العنقودية المترممة (٢٠,٦مم) والمكورة العنقودية البرازية (١٧,٧مم)، الاشريكية القولونية (٢٤,٦مم).

الاستنتاجات:

نتائج فعالية الزنجبيل ضد الميكروبات قورن مع نوع فعال من الأدوية لإيجاد نسبة معدل التثبيط المقرب.معدل التثبيط المقرب أظهر أعلى نسبة ضد المكورات العنقودية المترممة (٤٢,٣%) وأقل نسبة ضد المكورات العنقودية الذهبية٦,٣%).

Chapter one

Introduction

Rationale

Objectives

1.1 Introduction

Urinary tract infections are caused by microbes such as bacteria overcoming the body's defenses in the urinary tract. They can affect the kidneys, bladder, and the tubes that run between them. They are one of the most common types of infection and account for around 8.1 million visits to a doctor every year. The urinary tract can be divided into the upper urinary tract and the lower urinary tract. The upper urinary tract consists of the kidneys and the ureters, and the lower urinary tract consists of the bladder and the urethra. [(1)]

Bacterial infection of the bladder (cystitis) and kidney (pyelonephritis) is more frequent in women, and the incidence of infection increases with age. Factors that predispose to UTI include instrumentation (e.g., catheterization, cystoscopy), pregnancy, anatomic abnormalities of the genitourinary tract, and diabetes mellitus[(2).]

Most UTIs ascend through a portal of entry in the urethra. Most pathogens responsible for community-acquired UTIs are part of the patient's normal bowel flora. *Escherichia coli* is the most common isolate; in women, colonization of the vaginal and periurethral mucosa may antedate infection of the urinary tract. Bacteria with fimbriae capable of adherence to epithelial cells are more likely to cause UTIs, and persons whose epithelial cells bind these fimbriae may be at greater risk for infection. The longer and protected male urethra may account for the lower incidence of UTI in men. Motile bacteria may swim upstream, and reflux of urine from the bladder into the ureters may predispose to thwxfcrgte development of kidney infection [(2)].

Ginger is plant with leafy stems and yellowish green flower , the ginger spice come from roots of the plant ginger is native to wormer part of Asia commonly use to treat various types of problems. [(3)]

Have active ingredient can help fight infections: gingerol the bioactive substance in fresh ginger, also lower the risk of infections. Also may drastically lower blood sugars. In fact ginger extract can inhibit the growth of many deferent types of bacteria.[(4)]

Diabetes mellitus is a disease caused by deficiency or diminished effectiveness of endogenous insulin, it is characterized by hyperglycemia deranged metabolism and sequelae predominantly effecting the vasculature. [5]

1.2 Rationale

The use of natural or alternative medicines has increased markedly over the last few years .more and more older adult are using complementary and alternative medicine dietary supplement and herbal remedies without advice from a physician on the assumption that these substances will have beneficial effect .This study will be done to evaluate the effectiveness of ginger to eliminate the bacteria which can be used as preventive agent from urinary tract infection in diabetes patient and it was never done before in this area.

1.3 Objectives

1.3.1 General objective:

To detect antibacterial activity of ginger on different types of bacteria isolated from diabetic patient with urinary tract infections.

1.3.2 Specific objectives:

1. To detect the effectiveness of extraction of ginger on gram positive and gram negative bacteria.
2. To determine which type of bacteria that causes urinary tract infection can be eliminated by ginger extraction.

Chapter two

Literature Review

2 Literature review

2-1 Urinary tract infection

Urinary tract infections (UTIs) are the most common infections seen in outpatient practice [2]. 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 104–105/ml. Quantitative urine culture is, therefore, a necessity for diagnosis [6].

2-2 Fast facts on urinary tract infections

Women have a lifetime risk of over 50 percent of developing a urinary tract infection (UTI). Common symptoms include a strong, frequent urge to urinate and a painful and burning sensation when urinating. A UTI is usually diagnosed based on symptoms and testing of a urine sample. UTIs can be cured with 2 to 3 days of treatment.

2-2-1Causes:

Diagram of kidneys. The urinary tract is comprised of the bladder, kidneys, ureters, and urethra. The vast majority of urinary tract infections (UTIs) are caused by the bacterium Escherichia coli (E. coli), usually found in the digestive system. Chlamydia and mycoplasma bacteria can infect the urethra but not the bladder. UTIs are given different names depending on where they occur. For example:

- A bladder infection is called cystitis.
- A urethra infection is called urethritis.
- A kidney infection is called pyelonephritis.
- The ureters are very rarely the site of infection. [1]

Most organisms that cause UTIs come from the fecal and vaginal flora; E. coli is b far the most common pathogen in uncomplicated outpatient t0-UTI. Klebsiella and Proteus are less common. In young, sexually active women Staphylococcus

saprophyticus accounts for 5% to 15% of cases of cystitis. In patients who experience recurrent infections, have been instrumented, or have anatomic defects or renal stones, Enterobacter, Pseudomonas, and Enterococci are more commonly cultured. Candida species are frequently encountered in hospitalized patients who are receiving broad spectrum antibiotics and have a bladder catheter. Two other important nosocomial pathogens are S. Epidermidis and Corynebacterium group D2. In 95% of cases, UTIs are caused by a single organism. Patients with structural abnormalities are more likely to have poly microbial infections [(2)].

2-2-2 Risk factors

People of any age and sex can develop a UTI. However, some people are more at risk than others. The following factors can increase the likelihood of developing a UTI:

Sexual intercourse, especially if more frequent, intense, and with multiple or new partners, Diabetes, Poor personal hygiene, Problems emptying the bladder completely, Having a urinary catheter, Bowel incontinence, Blocked flow of urine, Kidney stones, Some forms of contraception, Pregnancy, Menopause, Procedures involving the urinary tract, Suppressed immune system, Immobility for a long period, Use of spermicides and tampons and Heavy use of antibiotics, which can disrupt the natural flora of the bowel and urinary tract [(1)].

2-2-3 Symptoms

- Woman with pain in the abdomen.
- Abdominal pains are a common symptom of UTIs.
- The symptoms of a UTI can depend on age, gender, the presence of a catheter, and what part of the urinary tract has been infected.

2-2-3-1 Common symptoms of a UTI include

Strong and frequent urge to urinate, Cloudy, bloody, or strong-smelling urine, Pain or a burning sensation when urinating, Nausea and vomiting, Muscle aches and abdominal pains and People with catheters may only experience fever as a symptom, making diagnosis more difficult.

2-2-3-2 Acute pyelonephritis

Acute pyelonephritis is a sudden and severe kidney infection. If an individual develops this condition they could also experience upper back and side pain, high fever, shaking, chills, fatigue, and mental changes. It is considered an emergency and should be evaluated by a doctor immediately if suspected.

2-2-3-3 Cystitis

If a person has a bladder infection, they could also experience low fever, and pressure and cramping in the abdomen and lower back.

2-2-4 Complication

Most UTIs are not serious, but some can lead to serious problems, particularly with upper UTIs. Recurrent or long-lasting kidney infections can cause permanent damage, and some sudden kidney infections can be life-threatening, particularly if bacteria enter the bloodstream in a condition known as septicemia. They can also increase the risk of women delivering infants that are premature or have a low birth weight. [1]

2-2-5 Prevention

There are several measures that can be taken to reduce the risk of developing a UTI:

Drink lots of water and urinate frequently, Avoid fluids such as alcohol and caffeine that can irritate the bladder, Urinate shortly after sex, Wipe from front to back after urinating and bowel movement, Keep the genital area clean, Showers are preferred to baths and avoid using oils, Avoid using a diaphra gm or spermicide for birth control, Avoid using any perfumed products in the genital area, Wear cotton underwear and loose-fitting clothing to keep the area around the urethra dry and individuals are advised to contact a doctor if they develop the symptoms of a UTI, especially if they have developed the symptoms of a potential kidney infection. [1]

2-2-6 Diagnosis

Diagnosis will usually be made after asking about the symptoms and testing a urine sample to assess the presence of white blood cells, red blood cells, and bacteria. A method of collecting urine called "clean catch" is used. This requires that a person

wash their genital area before providing a urine sample mid-flow. This helps to prevent bacteria from around the genital area getting caught in the sample. If a person has recurrent UTIs, a doctor may request further diagnostic testing to determine if anatomical issues or functional issues are to blame. Such tests may include:

Diagnostic imaging: This involves assessing the urinary tract using ultrasound, CT and MRI scanning, radiation tracking, or X-rays.

Urodynamics: This procedure determines how well the urinary tract is storing and releasing urine.

Cystoscopy: This diagnostic exam allows the doctor to see inside the bladder and urethra with a camera lens, which inserted through the urethra through a long thin tube. [1]

In men

UTIs in men are rare. The incidence for men under the age of 50 years is between 5 and 8 men in every 10,000. The risk of infection increases with age. When men contract a UTI, it will infect the same organs and areas as a UTI would in a woman. For men, however, the prostate is also at risk of infection. A man with a circumcised penis is less likely to get a UTI that a man who has not undergone circumcision. Treatment methods would be similar to those used to treat UTIs in women. [1]

2-2-7 Treatment

Woman drinking water drinking plenty of water helps flush out urinary tract infections and lowers the risk of future infection. As UTIs are normally caused by bacteria, they are most commonly treated with antibiotics or antimicrobials.

The type of medication and length of treatment will depend on the symptoms and medical history of the individual. The full course of treatment should always be completed for UTIs to make sure that the infection is fully clear, and to reduce the risk of antibiotic resistance. UTI symptoms can disappear before the infection has completely gone. Drinking lots of fluids and frequently urinating are always recommended for people who have UTIs as this helps to flush out the bacteria. A

variety of pain relief medications may be prescribed to alleviate pain. Applying a heating pad to the back or abdomen can also help. An uncomplicated UTI is one that occurs in an otherwise healthy person with a normal clear urinary tract. These can usually be cured with 2 to 3 days of treatment. A complicated UTI is one that occurs in a person who is weakened by another condition, such as pregnancy or heart transplant. Complicated UTIs tend to require longer periods of antibiotics, usually between 7 to 14 days. To cure a UTI that is caused by problems within the urinary system, the underlying issue needs to be found and corrected. If left untreated, these infections can lead to kidney damage. If the person is seriously ill, they may need to be admitted to a hospital to ensure that they take in sufficient fluids and receive the right medication. People may also need to go to the hospital if they are one of the following:

Pregnant and are otherwise ill, Older adults, People with cancer, diabetes, multiple sclerosis, spinal cord injury, or other medical problems, Individuals with kidney stones or other changes in their urinary tract, Recovering from recent urinary tract surgery and Recurrent infections in women.

Women who have recurrent bladder infections may be advised to:

Take a single dose of an antibiotic after sexual contact, Take a single, daily dose of an antibiotic for at least 6 months, Take a 2-to-3-day course of an antibiotic if symptoms reappear, Undergo vaginal estrogen therapy if they have already had menopause, Home remedies, There are a number of suggested remedies that people with a UTI can try at home and drinking fluids and urinating frequently can help flush bacteria from the body, and using a heated pad for short periods can help to relieve discomfort. [1]

2-3 Complicated Urinary tract infections Associated with diabetes mellitus

Diabetes mellitus is a major risk factor for urinary tract infections (UTIs) and is also associated with increased risk of certain complicated UTIs such as emphysematous pyelonephritis (EPN), emphysematous pyelitis (EP), emphysematous cystitis (EC),

abscess, and renal papillary necrosis (RPN). Such conditions are potentially life-threatening and require prompt evaluation and management.[7]

2-3-1 Pathogenesis

The increased frequency of UTIs in diabetic patients is likely due to several mechanisms including the presence of glycosuria, neutrophil dysfunction and increased adherence of the bacteria to uroepithelial cells. Factors that increase the risk of UTIs in diabetes include age, metabolic control, diabetic nephropathy, autonomic neuropathy and vascular complications.[7]

Emphysematous complications in the kidney or the bladder are likely to be due to the presence of organisms that rapidly ferment glucose and produce carbon dioxide. Impaired transport of metabolic end products due to impaired tissue perfusion in diabetes may also contribute. [8]

The pathogenesis of XGP is still obscure. In this condition, renal tissue is destroyed and replaced by hard, yellow xanthogranulomatous material. Suggested etiologies of XGP include chronic renal obstruction and infection, alterations in lipid metabolism, lymphatic obstruction, and renal ischemia. Patients with XGP commonly have diabetes or immunodepression. [10, 9]

Renal abscess in an uncommon infection of the urinary tract. It can develop by one of two general mechanisms: Hematogenous spread and ascending infection from the bladder. Diabetes mellitus is a risk factor for the development of renal abscess in association with ascending infection. Anatomical abnormality in the urinary tract such as vesicoureteral reflux and renal stones is usually present. Perinephric abscess usually occurs because of disruption of a corticomedullary renal abscess or an obstructing renal pelvic stone. [11-12]

Pathogenesis of RPN is presumed to be due to a marginal change in vascular supply leading to infarction and sloughing of papillae. Its etiology includes diabetes, analgesic abuse, sickle cell disease, pyelonephritis, renal vein thrombosis,

tuberculosis, and obstructive uropathy. More than half the patients with RPN have two or more of these causative factors. [13]

2-3-2 Emphysematous pyelonephritis

EPN is a severe, necrotizing form of multifocal bacterial nephritis with gas formation within the renal parenchyma. More than 200 cases have been reported in literature so far. [14].

Underlying poorly controlled diabetes mellitus is present in up to 90% of affected patients. [15]

The commonest offending organisms are Escherichia coli and Klebsiella followed by Proteus. The diagnosis of EPN is often delayed because the clinical manifestations are nonspecific and not different from the classic triad of upper UTI (i.e., fever, flank pain and pyuria). [15] Acute respiratory distress syndrome, disseminated intravascular coagulopathy, acute renal failure, disturbance of consciousness, and shock can reveal some severe forms.8 [15,14] Diabetic ketoacidosis is a very uncommon presentation, and only few cases has been reported so far.. [14,16,17]

EPN requires a radiological diagnosis. Conventional radiography may demonstrate gas bubbles overlying the renal fossa. Ultrasonography (US) characteristically shows an enlarged kidney containing high-amplitude echoes within the renal parenchyma. [18] Computed tomography (CT) is the imaging procedure osf choice to confirm the presence and extent of parenchymal gas.(15). A radiological classification has been proposed based on the location of the gas in the kidney as follows: Class 1: Gas confined to the collecting system, class 2: Gas confined to the renal parenchyma, class 3A: Perinephric extension of gas or abscess, class 3B: Extension of gas beyond the Gerota fascia and class 4: Bilateral or emphysematous pyelonephritis in a solitary kidney. [8]

2-3-3 Emphysematous pyelitis

EP is defined as the presence of gas localized to the renal collecting system. In contrast to EPN, only 59% of subjects had diabetes presumably due to a higher

proportion of patients with obstruction in this group. [19]. E. coli is again the most common organism. Patients have similar clinical symptoms to patients with noncomplicated pyelonephritis: Fever, nausea, vomiting, and abdominal pain. Leukocytosis and pyuria are observed in most patients. At conventional radiography, gas is seen filling and outlining the ureters and pelvicaliceal system. US or intravenous urography (IVU) may demonstrate an obstruction.[20]. CT best delineates gas within the collecting system and helps exclude complications, such as renal or perirenal fluid collections, frank abscesses, or EPN. [21]

2-3-4 Emphysematous cystitis

EC is a rare entity characterized by pockets of gas in and around the bladder wall produced by bacterial or fungal fermentation. More than 50% of patients with EC have diabetes mellitus. E.coli is the most common infecting organism.[22]

The clinical presentation of EC is nonspecific and ranges from incidental diagnosis on abdominal imaging to severe sepsis. Patients may complain of irritative symptoms, abdominal discomfort or hematuria. The presence of pneumaturia is a rare, although more specific, clinical finding. [23,24]

The radiographic findings provided the first and the only diagnostic clue. Nevertheless, CT is considered to be the preferred method of diagnosis because of its high sensitivity and specificity in the detection of abnormal gas and its anatomical extension. [25]

2-3-5 Xanthogranulomatous Pyelonephritis

XGP is a rare entity representing 1% of all renal infections.3 It most often occurs in middle-aged women with a history of recurrent UTIs. Two forms of XGP, a diffuse form (85%) and a focal form (15%), are well known. The typical presenting symptoms include flank pain, fever, malaise, anorexia, and weight loss. A unilateral renal mass can usually be palpated. Urine cultures most often reveal E. coli and Proteus mirabilis. Classic urographic triad in diffuse XGP consists of unilaterally decreased or absent renal excretion, a staghorn calculus, and a poorly defined mass or

diffuse renal enlargement. Sonography typically reveals echogenic calculi and multiple hypoechoic structures representing purulent loculations. [9,10] CT is the mainstay of diagnostic imaging for XGP and is helpful in demonstrating extension of the process into adjacent organs. The most frequent findings in the CT scan are calculi, hydronephrosis, kidney enlargement, expansion of the calices, renal pelvis contraction, and hypodense areas, with parenchyma destruction.[10,9] The final diagnosis of XGP is based on histology, usually after nephrectomy. [9]

2-3-6 Renal/perirenal abscess

A perinephric abscess is a collection of purulent material around the kidneys, with a presentation that is insidious. Diabetes is present in 30-40% of cases. [26] Presenting symptoms are often nonspecific. Only occasionally, a patient presents with a syndrome suggestive of acute pyelonephritis. The most common symptoms include fever, flank or abdominal pain, chills, dysuria, weight loss, lethargy, and gastrointestinal symptoms. A flank mass is palpable if the abscess is large or located in the inferior pole of the kidney space. The usual organisms include E. coli, Klebsiella, and Proteus species. IVU is often abnormal, but US or CT scan are the best means to establish the diagnosis of renal abscess. US or CT scan-guided aspiration of the abscess may then follow.

2-3-7 Renal papillary necrosis

RPN has a variable clinical course that ranges from a chronic, protracted, and relapsing form to an acute, rapidly progressive form. The acute progressive form is particularly rare, but the effects result in death from septicemia and renal failure. [27,28]. Patients with the more common chronic form may remain asymptomatic or symptomatic. The most common presenting symptoms in symptomatic patients include fever and chills, flank and/or abdominal pain, and hematuria. This condition should be suspected in diabetic subjects who develop recurrent episodes of UTI, renal colic, hematuria, obstructive uropathy, or unexplained renal failure. IVU is the most sensitive investigation for RPN, but it is rarely used today because of the adverse

effect of contrast media on renal function in diabetic patients. In patients with poor renal function, CT may demonstrate necrotic papillae allowing the diagnosis of RPN to be made. [15]

2-4 Management of complicated UTIs in diabetes

The initial management of a patient with EPN is resuscitation; a three-pronged approach should be put into place to address fluid/hemodynamic status, diabetic control and an antibiotic regimen. A decision must then be made as to whether medical therapy alone, percutaneous drainage or nephrectomy is required. Earlier series have stressed the need for urgent nephrectomy. With the advent of CT scanning, more powerful antibiotics, and better access to life support, an alternative conservative approach has emerged, based on appropriate antibiotics and percutaneous drainage. [15,29]

Nephrectomy is now limited to a select group of patients with EPN who are fit for surgery, and fulfill one or more of the following criteria: Possession of a nonfunctioning kidney; presentation of gross renal parenchymal destruction; existence of two or more risk factors (altered consciousness, thrombocytopenia, shock, and acute renal failure). Trials have been made with parental antibiotics and percutaneous drainage in classes 3A, 3B, and 4 in the absence of risk factors. [8,15]

Treatment of EP and EC involves broad-spectrum antimicrobial therapy, hyperglycemic control, and adequate urinary drainage with correction of any outlet obstruction. Patients with necrotizing infections will require more aggressive treatment that includes surgery. [24]

The gold-standard therapy for XGP is nephrectomy, which is total in the majority of cases. Nephrostomy before nephrectomy can be considered a method that facilitates surgery, because it allows a reduction in renal mass. Preoperative and postoperative broad-spectrum antibiotics and symptomatic management are also key factors for successful management of this condition. The development of antibiotics, advances in diagnostic modalities, and the introduction of nonsurgical intervention methods such

as percutaneous drainage and aspiration have all contributed to the improved outcome of renal and perirenal abscesses. With these changes, the rate of complete recovery from renal and perirenal abscesses without surgery has increased, and reduced mortality has been documented in several studies. [26]

Treatment of RPN includes aggressive antibiotic therapy when infection is demonstrated. Relief of obstruction may also be required. The prognosis for this condition is not well defined. [27,28]

2-5 Ginger

As one of the most used dietary condiments in the world today, it's no wonder that the benefits of ginger are pretty impressive. [30] It's versatile, easy to use and has been associated with everything from beating motion sickness to better brain function. The health benefits of ginger are largely due to its antioxidants, anti-inflammatory properties and content of therapeutic compounds like gingerol, shogaol, paradol and zingerone. The health benefits of ginger are well-documented and ginger has been used across the globe as a natural remedy for thousands of years due to its medicinal properties. It can be found in fresh, ground or capsule form — or even as ginger essential oil — and it's associated with an extensive list of ginger health benefits.

2-5-1 Scientific classification

Kingdom: Plantae

Clade: Angiosperms

Clade: Monocots

Clade: Commelinids

Order: Zingiberales

Family: Zingiberaceae

Genus: Zingiber

Species: Z. officinale

Binomial name: Zingiber officinale. [31]

2-5-2 Ginger Nutrition

Ginger contains a diverse array of many important vitamins and minerals. It also contains gingerol, a compound with potent antioxidant and anti-inflammatory properties that has been linked to many unique health benefits. [32]

100 grams (about 3.5 ounces) of raw ginger contains approximately [33]

80 calories.

17.8 grams carbohydrates.

1.8 grams protein.

0.7 grams fat.

2 grams dietary fiber.

415 milligrams potassium (12 percent DV).

0.2 milligrams copper (11 percent DV).

0.2 milligrams manganese (11 percent DV).

43 milligrams magnesium (11 percent DV).

5 milligrams vitamin C (8 percent DV).

0.2 milligrams vitamin B6 (8 percent DV).

0.7 milligrams niacin (4 percent DV).

34 milligrams phosphorus (3 percent DV).

0.6 milligrams iron (3 percent DV).

In addition to the nutrients listed above, ginger also contains a small amount of calcium, zinc, pantothenic acid, riboflavin and thiamin. However, keep in mind that most people consume a very small portion of ginger, so it should be combined with a variety of other nutrient-dense foods to meet your micronutrient needs.

12 Benefits of Ginger

1. Helps Treat Nausea

Used historically as a natural remedy for sea sickness and morning sickness, ginger is perhaps most well-known for its ability to treat nausea. One review looked at the

results of 12 studies comprised of 1,278 pregnant women and found that ginger was effective at decreasing symptoms of nausea with minimal risk of side effects.[34] Plus, another study from the University of Rochester Medical Center showed that ginger helped reduce nausea severity in patients receiving chemotherapy. [35]

2. Fights Fungal Infections.

Fungal infections cause a wide variety of conditions, from yeast infections to jock itch and athlete's foot. Fortunately, ginger has powerful anti-fungal properties that can safely and successfully help kill off disease-causing fungi. In one 2016 test-tube study out of Iran, ginger extract was found to be effective against two types of yeast that commonly cause fungal infections in the mouth.(36) Another test-tube study in Mycoses measured the antifungal effects of 29 plant species and found that ginger was the most effective at killing off fungus. [37]

3. Protects Against Stomach Ulcers.

Stomach ulcers are painful sores that form in the lining of the stomach and cause symptoms like indigestion, fatigue, heartburn and abdominal discomfort. Several studies have found that ginger could help prevent the formation of stomach ulcers. In fact, one 2011 animal study showed that ginger powder protected against aspirin-induced stomach ulcers by decreasing levels of inflammatory proteins and blocking the activity of enzymes related to ulcer development. [38]

4. Eases Menstrual Pains.

Unfortunately, adverse side effects like pain, period cramps and headaches are commonly associated with menstruation for many women. While some turn to over-the-counter medications to provide symptom relief, natural remedies like ginger can be just as useful at easing menstrual pain. A study published in the Journal of Alternative and Complementary Medicine showed that ginger was as effective at reducing menstrual pain as medications like ibuprofen and mefanamic acid. [39] Another study in 2009 had similar findings, reporting that ginger was able to reduce both the intensity and duration of pain. [40]

5. May Inhibit Cancer Growth.

One of the most impressive benefits of ginger is its anti-cancer properties, thanks to the presence of a powerful compound called 6-gingerol. Test-tube studies show that ginger and its components may be effective in blocking cancer cell growth and development for ovarian, pancreatic and prostate cancer [41,42,43]. However, more research is needed to determine how the anti-cancer properties of ginger may translate to humans.

6. Regulates Blood Sugar

High blood sugar can cause many negative symptoms, from frequent urination to headaches and increased thirst. If left unchecked, it can even cause more serious problems like nerve damage and impaired wound healing. Research shows that ginger may be able to help promote normal blood sugar to prevent these serious side effects. In one 2015 study, ginger supplementation actually reduced fasting blood sugar by 12 percent and improved long-term blood sugar control by 10 percent. [44]

7. Relieves Joint and Muscle Pain.

Because of its ability to reduce inflammation, adding ginger into your diet could help treat both muscle pain and arthritis-related joint pain. One study showed that daily consumption of ginger resulted in moderate-to-large reductions in muscle pain caused by exercise-induced muscle injury. [45] Another study found that ginger extract helped decrease knee pain and the need for pain medication in individuals with osteoarthritis.[46]

8. Lowers Cholesterol Levels.

From producing bile to manufacturing hormones, cholesterol is essential to overall health. However, high levels of cholesterol can build up in the blood, causing blockages and increasing your risk of heart disease.

One of the biggest benefits of ginger is its ability to naturally lower cholesterol levels to reduce your risk of heart problems. A study conducted at Babol University of

Medical Sciences actually found that ginger was able to significantly reduce bad LDL cholesterol and raise beneficial HDL cholesterol compared to a placebo. [47] An animal study also showed that ginger was nearly as effective in lowering cholesterol as atorvastatin, a medication commonly prescribed for high blood cholesterol.[48]

9. Improves Brain Function.

Neurodegenerative conditions like Alzheimer's disease and Parkinson's have been linked to oxidative stress and chronic inflammation in the brain. With its wealth of antioxidants and potent anti-inflammatory properties, ginger is believed to play an important role in the health of your brain. Several animal studies have found that ginger extract could protect against brain aging and cognitive decline. [49,50] Not only that, but a 2012 study also found that ginger extract helped improve cognitive function and attention in middle-aged women.[51]

10. Blocks Bacterial Infections

In addition to its antifungal properties, ginger boasts the ability to fight off bacterial infections as well. Pathogenic bacteria are common culprits behind conditions like urinary tract infections, pneumonia and bronchitis. According to one test-tube study, the compounds found in ginger could help inhibit the growth of certain strains of bacteria that cause gum disease.[52] Another test-tube study showed that ginger extract was effective against several strains of drug-resistant bacteria as well. [53]

11. Eases Inflammation.

Although inflammation can be a normal, healthy immune response to injury and infection, chronic inflammation is believed to be a major contributor to conditions like heart disease, obesity, diabetes and cancer. [54]

One review in the International Journal of Preventive Medicine noted that ginger extract may help inhibit the synthesis of certain markers of inflammation. Besides gingerol, it also contains other anti-inflammatory compounds like shogaol, paradol and zingerone. [55]

12. Promotes Proper Digestion

One of the most powerful ginger benefits is its ability to support digestive health and prevent problems like dyspepsia, a common condition of impaired digestion characterized by symptoms like pain, heartburn, fullness and discomfort. According to a study in the World Journal of Gastroenterology, ginger was able to speed up the emptying of the stomach by 25 percent compared to a placebo in people with indigestion. [56]

2-5-3 Precautions

In moderation, ginger is generally safe and unlikely to cause any adverse side effects in most people. Common symptoms reported include stomach discomfort, heartburn and diarrhea. When applied to the skin, ginger essential oil may cause skin irritation in some people. It's best to try a skin patch test by applying a small amount of oil first to make sure your skin is not sensitive. Additionally, if taking ginger capsules, always start with a low dose and work your way up to assess your tolerance. Stick to the recommended dosage and decrease as needed if you have any negative symptoms. [1]

2-5-4 Final Thoughts

Ginger is one of the most commonly used dietary condiments in the world. it may be effective at decreasing morning sickness and easing menstrual pains. Other ginger benefits for men and women include fighting fungal and bacterial infections [1].

2-6 Diabetes Mellitus

2-6-1 Definition and Epidemiology

As per the WHO, diabetes mellitus (DM) is defined as a hetrogeneous metabolic disorder characterised by common feature of chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism. DM is a leading cause of morbidity and mortality world over. It is estimated that approximately 1% of population suffers from DM. The incidence is rising in the developed countries of the world at the rate of about 10% per year, especially of type 2 DM, due to rising

incidence of obesity and reduced activity levels. DM is expected to continue as a major health problem owing to its serious complication.especially end-stage renal disease, IHD, gangrene of the lower extremities, and blindness in the adults. It is anticipated that the number of diabetics will exceed 250 million by the year 2010[56].

2-6-2 Classification and Etiology

The older classification systems dividing DM into primary (idiopathic) and secondary types, juvenile-onset and maturity onset types, and insulin-dependent (IDDM) and non-insulin dependent (NIDDM) types.

I. Type 1 Diabetes Mellitus (10%) (earlier called Insulin-dependent, or juvenile-onset diabetes):

a/ Type IA DM: Immune-mediated.

b/ Type IB DM: Idiopathic.

II. Type 2 Diabetes Mellitus (80%).

(earlier called non-insulin-dependent, or maturity-onset diabetes).

III. Other specific types of diabetes (10%).

A. Genetic defect of β-cell function due to mutations in various enzymes (earlier called maturity-onset diabetes of the young or MODY) (e.g. hepatocyte nuclear transcription factor—HNF, glucokinase).

B. Genetic defect in insulin action (e.g. type A insulin resistance) C. Diseases of exocrine pancreas (e.g. chronic pancreatitis, pancreatic tumours, post-pancreatectomy).

D. Endocrinopathies (e.g.acromegaly, Cushing's syndrome, pheochromocytoma)

E. Drug- or chemical-induced (e.g. steroids, thyroid hormone, thiazides, β-blockers etc).

F. Infections (e.g. congenital rubella, cytomegalovirus).

G. Uncommon forms of immune-mediated DM (stiff man syndrome, anti-insulin receptor antibodies)

H. Other genetic syndromes (e.g. Down's syndrome, Klinefelter's syndrome, Turner's syndrome.

IV. Gestational diabetes.

1 and type 2; besides there are a few uncommon specific etiologic types, and gestational DM. American Diabetes Aslsociation (2007) has identified risk factors for type 2 DM:

1. Family history of type 2 DM
2. Obesity
3. Habitual physical inactivity
4. Race and ethnicity (Blacks, Asians, Pacific Islanders)
5. Previous identification of impaired fasting glucose or impaired glucose tolerance.
6. History of gestational DM or delivery of baby heavier than 4 Kg
7.Hypertension.
8. Dyslipidaemia (HDL level < 35 mg/dl or triglycerides > 250 mg/dl)
9. Polycystic ovary disease and acanthosis nigricans.
10. History of vascular disease..

GESTATIONAL DM. About4% pregnant women develop DM due to metabolic changes during pregnancy. Although they revert back to normal glycaemia after delivery, these women are prone to develop DM later in their life. (56)

2-7 Previous studies

The study aimed to know the pathogens that infect the urinary tract and the study of their sensitivity to antibiotics as well as compare the effect of aqueous extract of ginger with the impact of antibiotics on the growth of bacterial isolates. Sixty three clinical urine samples were brought to microbiology laboratory at the Department of Community Health. The urine samples were cultured on the appropriate media and then carried out biochemical tests after that the Api 20 used to confirm the diagnosis. The results were showed isolation and identification of the bacterial isolates (*E.coli* 58.7%, *P. mirabilis* 28.5% and *K. pnumonae* 20.6%). Also the results of the use of

antibiotics (cefamandole (30mcg), streptomycin (10mcg), Piperacillin (10mg), Ceftizoxime (5mcg), rifampin (5mcg), the E.coli and P.mirabilis were resisted the influence of the antibiotics while the K. pnumonae was sensitive to cefamandole, streptomycin and resistant to other antibiotics. The results of the use of an aqueous extract of ginger (50% and 100%) concentration were showed the obvious effect on the growth of the bacteria isolated. [57]

Chapter three

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

3.1.3 Study population

Diabetic patients in Shendi hospitals.

3.2.3.1 Inclusion criteria

Diabetic patients in different ages with urinary tract infection.

3.2.3.2 Exclusion criteria

Specimens of Diabetic patients negative for bacterial urinary tract infection and under treatment were excluded.

3.1.4 Sample size

N=100sample

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.

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. [59].

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 smea.

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) [59].

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. [58].

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 4).

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 4).

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 4).

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 4).

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 4).

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 4).

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* [60].

Result : (Appendix 4).

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 [61].

Result: (Appendix 4).

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 4).

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 4).

3.1.13 Preparation of the extracts:

Extraction was carried out according to method descried by Sukhdev et. al. (2008):

The plant sample was coarsely powdered using mortar and pestle. Coarsely sample was successively extracted with petroleum ether using soxhelt extractor apparatus. Extraction carried out for about five hours till the colour of solvents at the last siphoning time returned colorless. Solvents were evaporated under reduced pressure using rotary evaporator apparatus. Finally extracts allowed to air in Glass container till complete dryness and the yield percentage were calculated as followed:

Weight of extract obtained / weight of plant sample X100

Sample	Weight of sample in gm	Weight of extract in gm or volume of the oil in ml	Yield %

Petroleum ether SD Fine India

Soxhlet Duran UK

Rotary evaporator Buchi Switzerland

Sukhdev. S. H; Suman. P. S. K; Gennaro. L and Dev. D. R (2008). Extraction technologies for medicinal and aromatic plants. United Nation Industrial Development Organization and the International Center for Science and High Technology. pp 116.

3.1.14 Procedure of inoculation in Mueller Hinton agar plates and applying ginger 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 36noculums. The excess 36noculums 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 ginger extract by using automatic pipette in volume 50 µ.l of concentrations 100, 50, 25, 12.5 mg/ml.
6. The plates were incubated for 24h in incubator under aerobic condition in 37ºC.

3.1.15 interpreting the sensitivity of ginger 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 ginger 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 chloramphnequle 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 Ginger leaves

Dried ginger 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 [61].

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 four

Results

5. Results

In this study 100 sample collected from diabetic patient was suffering from UTI, 62% was growth and 38% no growth.

4.1 Frequency and percentage of sampling recording the age group:

Table 1 Frequency and percentage of sampling recording the age group ' age group frequency and percentage.

Age group	Frequency	Percentage
30 – 40	10	16.13
41 – 50	16	25.81
51 – 60	17	27.40
61 – 70	14	22.60
71 – 80	4	6.45
> 80	1	1.61
Total	62	100%

4.2: Bacteriological result.

4.2: Gram stain for isolated bacteria species.

Out of the 62 positive cultures for bacterial growth 42 were gram positive (67.7%) and 20 gram negative (32.3%) are shown in table 2.

Gram reaction	Frequency	Percentage
Gram positive	42	67.7
Gram negative	20	32.3
Total	62	100%

Table 3: Frequency and percentage of isolated bacteria.

Bacteria isolated	Frequency	Percentage
S.aureus	18	29
S.saprophyticas	18	29
E.fecales	6	9.7
E.coli	20	32.3
Total	62	100%

Table 4: Mean of inhibition zone diameter for isolated organisms in different concentration (100mg/dl, 50mg/dl, 25mg/dl, 12.5mg/dl) of Ginger extraction.

Conc organisms	100	50	25	12.5
S.aureus	7.8	8.2	8.1	9.6
S.saprophyticas	13.4	10.7	10.1	10
E.fecales	7	7	10.6	10.3
E.coli	9.5	8.5	7	7

Table 5: Show antimicrobial susceptibility of ginger extract compared to chloramphenicol.

Organism	Mean of inhibition zone diameter (mm)	
	Ginger extract 100 mg/ml	Positive control chloramphenicol
S.aureus	7.8	31
S.saprophyticas	13.4	20.6
E.fecalis	7.0	17.7
E. coli	9.5	24.6

Table 6: Show the relative percentage inhibitions of ginger extract compared to chloramphenicol.

Test organism	Relative percentage inhibition
S.aureus	6.3%
S.saprophyticas	42.3%
E.fecalis	15.6%
E.coli	14.9%

Chapter five

Discussion
conclusion
Recommendations

5.1 Discussion

In the study Hundred sample collected from diabetic patients were suffering from UTI (62%) were growth. The main causative agent of UTI in the study population was *E.coli* (32.3%) it has been reported to be the most frequent pathogen among diabetic pt. This is in agreement with report form Prof Khalid- The results were showed isolation and identification of the bacterial isolates *E.coli* (58.7%). [57] Followed by *S.aureus* and *S.saprophyticus* (29%), and the last *E.fecalis* (9.7%). In study antimicrobial susceptibility of ginger extract show highest zone of inhibition against *S.saprophyticus* (13.4mm) and lowest zone of inhibition against *E.fecalis* (7mm), *E.coli* (9,5mm), *S.aureus* (7.8mm).

The result show that the extract of ginger have an antimicrobial activity against both gram positive and gram negative bacteria, this is agreement with study of NADA mentioned that :the extract of ginger have an antimicrobial activity against both gram negative and gram positive bacteria .this may be caused as a result of the presence of gingerol and shogaol as active ingredient within ginger. Also the results for extract in different concentrations were more effective against the gram positive bacteria compared to the result for gram negative, The higher resistance of the gram – negative bacteria could be due to the complexity of the cell wall of this group of microorganism .Indeed ,the external membrane of gram –negative bacteria renders highly hydrophilic surfaces whereas the negative charge of the surface of gram – positive wall may reduce their resistance to antibacterial compound [62]

The results of antimicrobial activity of ginger extract was compared with positive control (standard drug) for evaluating their relative percentage inhibition while the extract exhibit maximum relative percentage inhibition against *S.saprophyticus* (42.3%)and minimum relative percentage inhibition against *S.aureus* (6.3%).

The highest inhibition zone of ginger extract13.4mm in diameter against *S.saprophyticus* at concentration 100%, followed by *E.coli* 9.5mm ,7.8mm against *S.aureus* and 7mm against *E.fecalis*. Mohmoud M. Elaasser found that ginger have antimicrobial activity against bacteria (2016). [63]

5.2 Conclusion

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

Lowest concentration of ginger extract able to inhibit the growth of bacteria cause UTI 12.5mg/dl.

Inhibitory effect of ginger extract in both gram negative and gram positive bacteria.

5.3 Recommendations

1. Making more studies about effect of ginger extract on bacterial infection happens in other body systems.
2. Data from in vitro studies on the antimicrobial effects of ginger are promising, but human data are currently lacking. Therefore, it is essential to have in vivo studies on antibacterial effects of ginger.
3. Human clinical trials also need to evaluate the synergistic effect between ginger and antibiotics used in UTIs and evaluated the efficacy of its gingeriol in the treatment of UTIs in the future.

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Appendices

Appendixes 1: Questionnaire

University of Shendi

Faculty of medical laboratory sciences

College of Graduate Studies

Detection of antibacterial of ginger extract of bacteria Isolated from Diabetic Patients with Urinary Tract Infection

Name……

State……

City……

Village……

1- Age:

a- 30- 40 (……) c- 41 – 50 (……). d- 51 – 60 (……...).

e- 61- 70 (…..) f- > 80 (……).

2- Gender

a- male (……) b- Female (……)

3- Diabetes Treatment:

a- Tabs (……). b- Insulin (……).

4- History of urinary tract infection in previous……

5- UTI treatment……

6- Response to treatment:

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

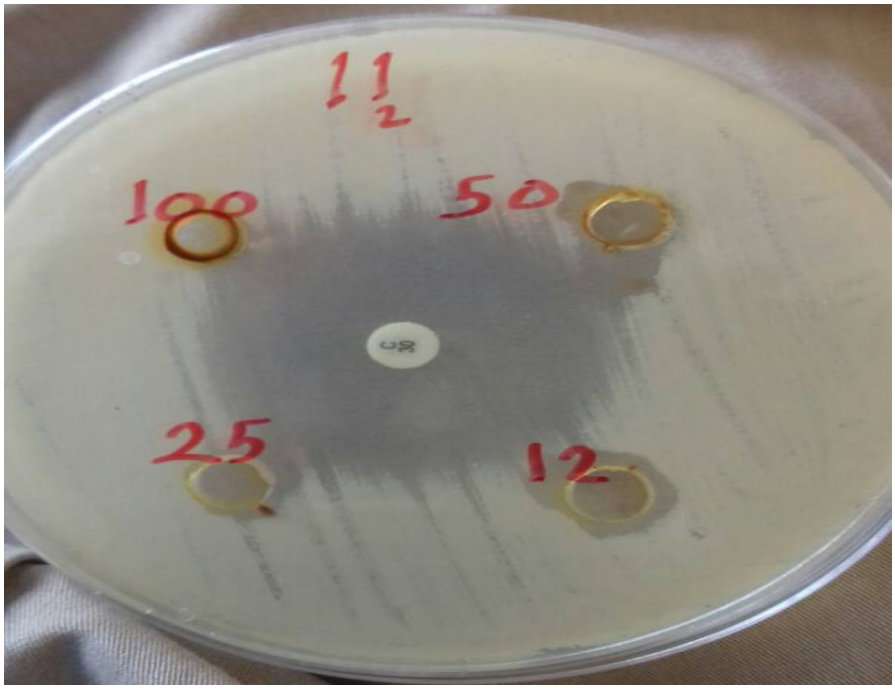
7- Use of herbal

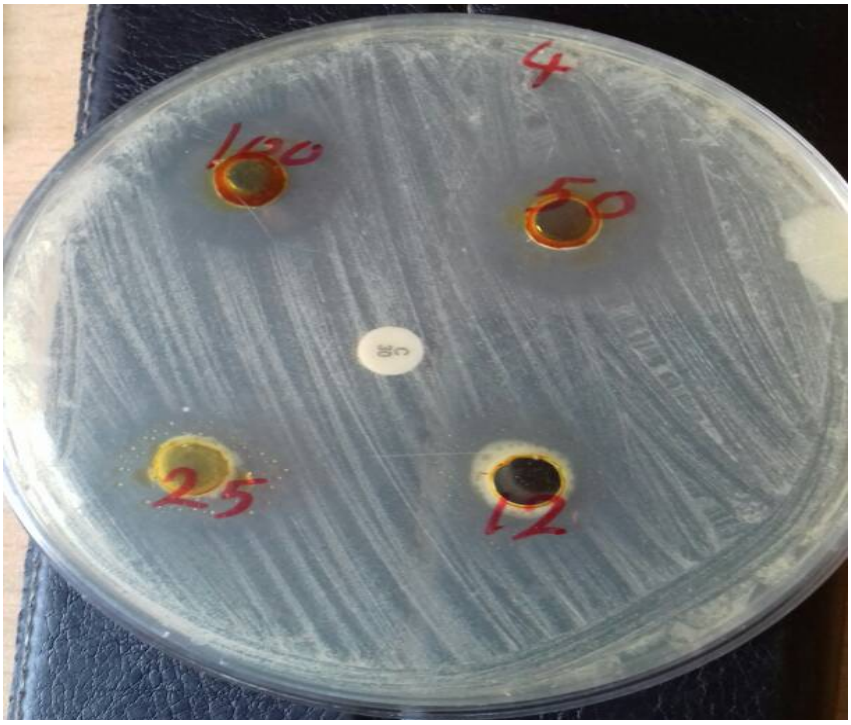
a- yes (……). b- no (…….).

8- Other disease:

a- Other (……) b- No (……)

Appendixes 2: Plate: ginger extract inhibition zone in control strain *S.aureus*.





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	-	-	+	-	+	-	+	+	+

Biochemical reactions of isolated Gram positive cocci

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

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	-	+	+	-	+	+