Sudan University of Science and Technology

College of Graduate Studies

Antibacterial Activity of *Adansonia digitata L* and *Tamarindus indica* against Selected Isolates from Diabetic Patients with Recurrent Urinary Tract Infections in Al-Faysial Hospital

فعالية مستخلصي التبلدي والعرديب كمضاد للبكتيريا المعزولة من التهاب المسالك البولية المتكررة لدي مرضي السكري بمستشفي الفيصل

A Thesis submitted in partial fulfillment for the requirement of M.Sc. in Medical Laboratory Science (Microbiology)

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قال تعالى:

تَبَارَكَ الَّذِي بِيَدِهِ الْمُلْكُ وَهُوَ عَلَى كُلِّ شَيْءٍ قَدِيرٌ)

صدق الله العظيم

سورة الملك الاية ١

DEDICATION

To my guardian angels my parent,

Beloved Brothers, sisters,

Supportive colleaguesand friends.

2. AKNOWLEDGMENT

All thanks and praise to ALLAH the lord of all worlds for all givens and blessings rewards to me. With sincere thanks and grateful, I would like to acknowledge my supervisor **Dr/ Ahamed Ibrahim Hashim** for this outstanding, knowledge encouragement, guidance, patience and constructive advice throughout this work. Special thanks to **Dr/Aboalgasim**and**Ust/ Muduther****Abdalrahim for**their contribution in analysis Statistical Package for the Social Sciences (SPSS)

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ABSTRACT

This study was aimed to validate the antibacterial activity of different concentration of methanol extracts *Tamarindus indica* fruit (50% , 25%, 12.5%, 6.25%) and *Adansonia digitata*fruit (100% ,50%, 25%, 12.5%) against recover resistant isolates of recurrent urinary tract infection among diabetic patient in Khartoum state. The study was conducted during period of May to August 2015.

Patients with diabetes using different protocol based to control diabetes were enrolled in this study. Pretested structured questionnaire was used for collection of demographic data from each patient. Urine samples were collected from both sex (58 males and 42 females). The Urine samples were inoculated directly in Cystine lactose electrolyte deficient under aseptic condition, and transported directly to the Research Laboratory of Sudan University of Science and Technology for processing. The bacteria were identified by Gram stain and biochemical tests. Out of 100 urine samples investigated, only (85%) samples revealed positive bacterial growth in Cystine lactose electrolyte deficient. The identified species were*E.feacalis* (5.6%), *S.aureus* (20.4%) , *S.saprophyticus* (4.5%) *E.coli* (25%) , *K.pneumoniae* (17 %) ,*P.aeruginosa*(6.8%) , *P.vulgaris* (5.7%) and Resistant were :*S.aureus* 4(18.1%) , *E.coli* 12 (54%) , and *Ps.aeruginosa* 4 (18.1%) .

In this study *E.coli* strains were susceptible to Aradeb extract more than Tebaldi Extract which shows weak activity, in comparsion to *S.aureus* strains and *P.aeruginosa* strains which activity of extracts were equals.

4. مستخلص الاطروحة

قد بدأت هذه الدراسة للتحقق من النشاط المضاد للبكتيريا للمستخلصي الميثانول لثمرة العرديب والتبلدي ضد البكتريا المقاومة للمضادات الحيوية المعزولة من عدوى المسالك البولية المتكررة لدي مرضي السكري في ولاية الخرطوم. وقد أجريت الدراسة خلال الفترة من مايو حتي أغسطس العام ٢٠١٥ . في هذة الدراسة يستخدم مرضى السكري طرق مختلفة لتنظيم مرض السكر، تم استخدم جمع البيانات الديموغرافية من كل المرضى بأستخدام أستبيان تم أعداده مسبقا .

تم جمع عينات البول من كلا الجنسين (٥٨ ذكور و ٤٢ إناث). تم زراعة عينات البول مباشرة في وسط أذراع بمزرعه الستستين الشحنات ناقصة اللاكتوز تحت ظروف معقمة، ونقلت مباشرة إلى مختبر أبحاث جامعة السودان للعلوم والتكنولوجيا للمعالجة. وقد تم التعرف على البكتيريا موجبة الغرام وسالبة بواسطة صبغة غرام بالأضافة للأختبارات البيوكيميائية.

من أصل ١٠٠ عينة بول ، أظهرت فقط (٨٥%) عينة نموءا إيجابيا البكتيري في سيستين اللاكتوز ناقصة الشحنات ، حيث كانت الأنواع التى تم التعرف عليهاالمعوية البرازية(٥.٦%)، المكورات العنقودية الذهيبية (٢٠.٤%)، المكورات العنقودية المترممة(٤.٥%)،الإشريكية القولونية(٢٥%)،الكلبسيلة الرئوية(١٧%)،الزائفة الزنجارية(٦.٨%)،،المتقلبة الاعتيادية(٥.٧%) و. حيث كانت البكتريا المقاومة للمضادات الحيوية هي المكورات العنقودية الذهيبية ٤ (١٨.١%) والإشريكية القولونية ١٢(٥٤%) ،الزائفة الزنجارية ٤ (١٨.١%).

في هذه الدراسة سلالة الإشريكية القولونية كانت أكثر تأثرا بمستخلص العرديب أكثر من مستخلص التبلدي الذي أظهر نشاطا أقل ، مع مقارنتة بسلالاتالمكورات العنقوية الذهبية والمتقلبة الأعتيادية حيث كان نشاط مستخلصي العرديب والتبلدي متساويا.

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LIST OF ABBREVIATIONS

CLED Cystine lactose electrolyte deficiency

DNAse Deoxyribonuclease

FDA Food drug administration

MDR Multi drug resistant

MIC Minimum inhibitory Concentration

MRSA Methacillin Resistant *S.aureus*

RUTI Recurrent urinary tract infection

SGLT2 Sodium-glucose cotransporter-2

UTI Urinary tract infection

WHO World Health Organization

ZDIZone diameter of inhibition

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1. Introduction

The range of pathogenic bacteria is wide so the variety of diseases caused by them. Despite the existence of potent antimicrobial agents, resistant or multi-resistant strains are continuously emerging, imposing the need for a continuous search and development of new drugs (Barbour *et al.*, 2004; Machado *et al.*, 2003) , as UTI-causing bacteria became more resistant to available antibiotics, the need to explore new strategies for managing UTIs is clear (Foxman, 2010),this led to increase urgency for new intervention with availability , low cost , more effectiveness as antibacterial and aware about medicinal plants and their therapeutic potential against pathogenic bacteria, particularly to those are at risk of getting infections such as diabetic patients who are predisposed to drug or multidrug resistance then common or recurrent infections addition to have more antibiotic treatments compared with other subjects, which can increase the resistance rates in the bacteria(Saber *et al.*,2010).

Highest resistance rates in patients with diabetes mellitus are usually observed in countries, areas or hospitals where the compliance to the national antibiotic policy is low (Boyanova and Mitov, 2013).

WHO estimated that 80% of population of developing countries rely on medicinal plants or plant based drugs, for primary health care, some of medicinal plant such as *Adansonia digitata* and *Tamarindus indica* .

Phytochemical studies have shown that plants with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids and saponins*A. digitata* and *T. indica* have antimicrobial activity (Doughari, 2006)

1.2 Rationale

The use and misuse of antimicrobial drugs accelerates the emergence of drug-resistant strains, Poor infection control practices, inadequate sanitary conditions and inappropriate food-handling encourage further spread of antimicrobial resistance (WHO 2015).

Urinary tract infection is the most common bacterial infection encountered in clinical practice (Platt and Keating, 2007). Adults patient with diabetes are more susceptible to developing recurrent UTI due to various predisposing factors, such as hyperglycemia-related impairment of the immune response and glucosuria (Stapleton, 2002).

Also frequent use of antibiotics, such as quinolones, which are increasingly inactive against these organisms, contributes to the over-growth of bacteria in the gastrointestinal tract and their appearance in the genitourinary tract (Nicolett*i et al.*,2010).As UTI-causing bacteria become more resistant to available antibiotics; it is essential to explore new strategies for managing UTIs and find novel alternatives (Foxman, 2010).Medicinal plants could be a suitable alternative as they are effective, available, with affordable cost and minimal toxicity.

1.3 Objectives

The objectives of this research were;

1.3.1 General objective

To determine the antimicrobial activity of *Adansonia digitata* and *Tamarindus indica* against selected isolates from diabetic patients with recurrent urinary tract infections in Khartoum State

1.3.2 Specific objectives

a) To collect, isolate, identify and determine the Antibacterial sensitivity of clinical isolates against selected antibiotics.

b) To determine the Antibacterial potential of selected clinical isolates against *Adansonia digitata* and *Tamarindus indica*

CHAPTER TWO
LITRETURE REVIEW

2. Background

Herbal drugs have found wide spread use in many countries because they are easily available, cheaper and safer than synthetic drugs (Retnam and Britto, 2007). Antimicrobial resistance is a major and increasing global healthcare problem, a large number of bacteria have responded to the use of antibiotics with their ability to evolve and transmit antimicrobial resistance to other species, increased consumption of antimicrobial agents and inappropriate use can accelerate this phenomenon (Levyand Marshall , 2004).

Also the continuous migrations of people play an important role in acquisition and spread of Multi drug resistantstrains (Spellberg*et al.*, 2013). Sudanese medicinal plants have been reported to exert antimicrobial activity against viruses, bacteria and protozoa ,encourage further more research, with more benefits for patient been safe and effective as antibacterial with consider of drug resistance strains (Khalid*et al* ., 2012). *Tamarindus indica* and *Adansonia digitata* are common beverages in Sudan associated with social ceremony. In Asia *Tamarindus indica*fruit were used as source of flavor to food (Daniyan andMuhammad, 2008)

2.1*Adansonia digitata &Tamarindus indica L.*

Adansonia digitata wasnamed upon French botanist Michel Adanson (1727-1806), who lived in Senegal for 6 years and wrote a work on that country's natural history. Linneaus dedicated the genus and species to him; 'digitata' means hand shaped, referring to the shape of the leaf.(yagoub , 2008)

Is known by a very large number of local names: English (Baobab, Monkey bread tree, Ethiopian sour gourd, Cream of tartar tree, Senegal calabash fruit, Upside-down tree, French (pain de singe, arbre aux calebasses), Portuguese (Cabaçevre), Arabic (Buhibab, hamao-hamaraya, Habhab, Hamar and Tebaldi), Afrikaans (Kremetart), Hausa (Kuka), sotho (Seboi), tswana (Mowana), Tsonga (Shimuwu) and venda (Muvhuyu) (Rahul, 2015) .*Tamarindus indica* is leguminous tree of the genus *Tamarindus* is medieval latinization of the Arabic name for the fruit, meaning Indian date. The fruits of tamarind were traded widely in ancient times. (Tariq *et al.*, 2013) .

Records from the eastern Mediterranean showed*Tamaindus indica* was already in cultivation there in the fourth century B.C.(Before Centuery) On encountering the fruit in western India, Arab sea traders thought the sticky black pulp and seeds of the fruit resembled their native date palm, so they combined their common name for date palm 'Tamr', along with the Arabic name for India ('hindi'), to arrive at the common name tamrhindi on which the scientific name *Tamarindus* is based (Wallis, 2005). Arab were introduced it into Europe

2.2 *Adansonia digitataL*

2.2.1 Taxonomic Classification

Kingdom: Plantae

Subkingdom: Viridiplantae

Division: Tacheophyta

Super division: Spermatophyte

Class: Magnoliopsida

Sub-class: Rosana

Order: Malvaless

Family: Malvaceae

Genus: *Adansonia* (Sundarambal , 2015)

2. 2.2 Description of *Adansonia digitata* and distribution

Diameter of 10–12 m and a height of 23 meter or more, fruit capsule is covered with velvety hairs, can reach 12 cm and contains many seeds (Rahul, 2015). Recently, the European Commission authorized the importation of baobab fruit pulp as a novel food for human consumption (Buchamann*et al.*, 2010).

In 2009, it was approved by the Food and Drug Adminstration (FDA) as a food ingredient in the United States of America (Addy, 2009).

In central Sudan particular north kordofan it consider one of the cheapest source of food (El tahir*et al.*, 2010) furthermore its provide ecosystem services and environmental benefits, carbon sequestration biodiversity conversation, soil enrichment air and water quality (Jose, 2009*)*.

2.2.3 Chemical constituent

Recently, *A.digitata* has been referred to as a "super fruit" based on its nutritional profile (e.g. vitamin, fatty acid, mineral) and its fruit was found to contain 337mg/100 g of ascorbic acid (Gebauer*et al.*, 2002).

The calcium content reported 344.2 mg/100 g.Similarly, the level of potassium in the fruit pulp was found to be 1578.5 mg/100 g sample and1240.0 mg/100 g. (Osman, 2004)Proximate analysis of ripe fruit shows an average of 8.7% moisture with 2.7% protein, 0.2% fat, 73.7% carbohydrate, 8.9% fibers and 5.8% ash (Kamatou, 2011) .

The pulp sweetness is provided by fructose, sucrose and glucose contents. Fruit pulp is acidic due to the presence of organic acids including citric, tartaric, malic, succinic as well as ascorbic acid and the energy value of pulp is similar to that of baobab leaves (Becker, 1983).

Several classes of compounds have been identified from various parts of baobab (fruit pulp, seed oil, leaves, and roots) including terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids (Kamatou, 2011).

The presence of organic acids such as citric, tartaric, malic, succinic and ascorbic acid in the fruit pulp was first highlighted in the early fifties.

The pulp represents 14 to 28% of the total fruit weight and the pulp water content is low less than 15% (Soloviev et *al*., 2004). Studies have shown that the fruit pulp contains high amounts of carbohydrate (70%), crude fiber (11.2%), a low amount of ash (5.7%) and protein (2.2%), and a very low amount of fat (0.4%) (Lockett *et al*. , 2002). Several amino acids such as alanine, arginine, glycine, lysine, methionine, proline, serine, valine (from fruit pulp) vitamins (B1, B2, B3, A, C) (from fruit pulp and/or leaves) (UNCTAD, 2005) and minerals (Cu, Fe, K,Mg, Mn, Na, P, Zn) from fruit pulp have also been identified (Kamatou , 2011).

2.2.4 Uses

A.digitata have various medicinal usage anti- Sickling Activity, anti-diuretic , anti-diabetic, antibacterial , anti-oxidant , anti-inflammatory, anti-tyroponsal , hepato protective , Analgesic and Anti- Pyretic Activity and enhance drug permeation(Sundarambal*et al.*, 2015).

2.2.5 Antibacterial activity:

A study was conducted bylagnika*et al.*, 2012 were tested *A.digitata*against*Escherichia coli*CIP53126, *Staphylococcus aureus*ATCC6538, *Enterococcus faecalis*ATCC29212, *Pseudomonas aeruginosa*CIP82118,*Salmonella abony*CIP8039, MethicillinResistant*Staphylococcus aureus*(MRSA)and *Staphylococcus epidermidis.*Were the most sensitive found *S.aureus*; methicillinresistant *S.aureus*(MRSA) and *S.epidermidis.*

Also Magrate*et al.*, 2012 were evaluated antimicrobial activity *A.digitata*Methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*and*Candida albicans.*Screening revealed thatextract wereactive against*B. cereus*, MRSA, *P. aeruginosa*and *C. albicans*.

Djeussi*et al.*, 2013 were observed with the crude extracts of *A. digitata*, against *E. coli* MC4100 and *K. pneumoniae* KP55 with the ratios minimal bactericidal versus minimal inhibitory concentrations (MBC/MIC) equal to 1 and 2 respectively. *A. digitata* displayed the most important spectrum of activity

2.3 *Tamarindus indica*

2.3.1Taxonomic Classification

Kingdom: Plantae

Subkingdom: Tracheobionta– Vascular plants

Division: Tacheophyta

Super-division: Spermatophyta

Class: Magnoliopsida

Sub-class: Rosidae

Order: Fabales

Family: Fabaceae

Genus: *Tamarindus L* (Santosh , 2011)

2.3.2 Description and distribution of *Tamarindus indica*

It is a large tree, attaining 60 - 80 feet in height and bearing a very large, widely spreading head of foliage, trunk with a dark rough bark, youngest twigs smooth or slightly pubescent; flowers are in bunches, yellow in color and boat shaped; seeds are reddish brown, thick; the flattened sides of the seeds are marked by a centrally placed dull area; fruit pulp occurs as a reddish-brown, moist, sticky mass, in which yellowish-brown fibers are readily seen; odor is pleasant, taste is sweetish and acidic; bark of the trunk is scaly; leaves are par pinnate up to 15 cm long (Bentley, 2004).

Commonly spread and Africa to Senegal in west, Sudan south Ethiopia in east, Mozambique and Madagascar (Havinga, 2010) .It is also thought that the plant came to India from Africa (Bhadoriya*et al.*,2010)

According to World Health Organization report, tamarind fruit is an ideal source of all essential amino acids except tryptophan (82%) (Glew*et al.*, 2005).

2.3.3 Chemical constituents

Phytochemical investigation carried out on *T.indica* revealed the presence of many active constituents, such as phenolic compounds, cardiac glycosides,tartaric acid, the mucilage and pectin, arabinose, xylose, galactose, glucose, and uronic acid (Coutino*et al.* , 2001).

The pulp contains organic acids, such as tartaric acid, acetic acid, citric acid, formic acid, malic acid, and succinic acid; amino acids; invert sugar (25-30%); pectin; protein; fat; some pyrazines (trans-2-hexenal); and some thiazoles (2-ethylthiazole, 2-methylthiazole) as fragrant .Total protein; lipids with fatty oils; and some keto acids (Hänsel*et al.*, 1992). In the leaves of the plant, two triterpenes, lupanone and lupeol were found (Imam *et al.*, 2007).

2.3.4 Antibacterial activity

Study were done by Kothariand Seshadri , 2010 found that *T.indica* active against both gram-positive and gram-negative organisms. MIC values of potent extracts against susceptible organisms ranged from 53-380 µg/mL.

Routhu*et al.*, 2015mentioned that antibacterial Screening assay of *T.indica*displayed broadspectrumagainst two Gram-positive (*B.subtilis*and*S.aureus*) and threeGram negative (*K. pneumoniae*,*P. vulgaris*and *E.coli* . Likewise Anu*et al.*, 2014 were evaluatedmethanol crude extract of *T. indica* pulps*in vitro*to determine their inhibition activities against human pathogenic microorganisms *Bacillus subtilis* , justified that to presence of alkaloids and tannins.

Furthermore Ugoh*et al.*, 2013 reported that extracts of the fruits and leaves of *Tamarindus indica* were tested on *S. aureus*, *E. coli*, *S. typhi*

andPs. aeruginosa. The result exhibited by fruit extracts is a higher antibacterial activity than the leaf extracts.

Chowdhury*et al.*,2013 were showed more promising result they were demonstrate activity of *T. indica* and eleven other medicinal plant by disc diffusion against clinical isolate . *T. indica* was remarkable activity in compare to other, exhibited highest antimicrobial activity was found up to 80% in *Tamarindus indica* in case of *K. pneumoniae,Ps. aeruginosa* and *P. mirabilis* isolates.

2.4 Recurrent urinary tract infection in diabetic patients

Recurrent UTI is defined as uncomplicated UTIs in 6 month s or, more traditionally, as ≥3 positive cultures within the preceding 12 months.

Most recurrences occur within the first months after the primary infection, and there can often be clustering of infections. When the initial infection is caused by *E. coli*, there is a higher risk of reinfection within the first 6 months. When there is recurrent infection with the same organism despite adequate therapy, it is considered a relapse.

Classic symptoms of acute lower UTI include dysuria, urinary frequency and suprapubic pain plus or minus hematuria. Differential diagnoses include vaginitis, acute urethritis, interstitial cystitis, and pelvic inflammatory disease (Annette and Annick, 2010).

The risk of recurrent urinary tract infection (RUTI) is higher in diabetics compared to non-diabetics. The etiology and the antibiotic resistance of uropathogens have been changing over the past years. Predisposition to UTIs in diabetes mellitus results from several factors.

Susceptibility increases with longer duration and greater severity of diabetes (Chen *et al.*, 2009). High urine glucose content and defective host immune factors predispose to infection; hyperglycemia causes neutrophil dysfunction by increasing intracellular calcium levels and

interfering with actin and, thus, diapedesis and phagocytosis. Vaginal candidiasis and vascular disease also play a role in recurrent infections.

Recently, the use of SGLT2 (sodium-glucose cotransporter-2) inhibitors have led to a small but significant increase in urinary tract infections in patients with inadequately controlled diabetes mellitus. Levels of urinary glucose increased with greater doses of the medication; however, the incidence of urinary tract infections did not. The severity of infections was mild to moderate and responded to the administration of appropriate antibiotics (Johnsson*et al.*, 2013).

The deterioration in antibiotic efficacy threatens a return to the medical landscape of 50 years ago when few, if any, effective antimicrobial agents existed.

With emergence of antimicrobial resistance begin when exposure of a sensitive microbial population to an antibiotic leads to selection of resistant clones. This is followed by expansion of these clones which can, in turn, lead to an outbreak and epidemic or a pandemic. Imprudent use due to profligate prescribing practices and over-the-counter sales make the development of resistant strains much more likely. Once established, this resistance may not be reversible, necessitating the need for new antimicrobial agents or control strategies (Wagenlehner *et al.*, 2014).

Finally, many women experience frequent RUTIs, designated as either a "relapse" after treatment cessation with the pretherapy isolate or as a "reinfection" with a different organism after initial treatment cessation.

Although considered to be a benign condition, RUTI can have a significant impact on quality of life (Foxman, 2002).

Urinary tract infection can have higher prevalence of resistance to antimicrobials including ciprofloxacin, cephazolin, and nitrofurantoin (Ikram*et al.*, 2015).

WHO has reported Resistance to one of the most widely used antibacterial drugs for the oral treatment of urinary tract infections caused by *E. coli* – fluoroquinolones – is very widespread (WHO, 2015).

2.5Bacteriacausing Urinary tract infections

Most commonly by Escherichia coli,*Pseudomonas aeruginosa*,*Enterococcus faecalis*, *Proteus mirabilis*, *Staphylococcus saprophyticus*, *Staphylococcus aureus* and *Klebsiellapneumoniae*; with high recurrence rates and increasing antimicrobial resistance among uropathogens (Ana *et al.*, 2015).

2.5.1 *Escherichia coli* :

Escherichia coli is the most common and important member of the genus *Escherichia.* This organism is associated with a variety of diseases, including gastroenteritis and extra intestinal infections such as urinary tract infections (UTIs), meningitis, and sepsis. A multitude of strains are capable of causing disease, with some serotypes associated with greater virulence (e.g., *E. coli* O157 is the most common cause of hemorrhagic colitis). As pathogen illustrated by the fact the bacteria are : the most common gram-negative rods isolated from patients with sepsis , responsible for causing more than 80% of all community-acquired UTIs, as well as many hospital-acquired infections, and a prominent cause of gastroenteritis in developing countries.

E. coli is Gram-negative rod , facultative anaerobic rod, Fermenter; oxidase negative,Lipopolysaccharide consists of outer somatic O polysaccharide, core polysaccharide (common antigen), lipid A (endotoxin) and Virulence factors.

At least five different pathogenic groups cause gastroenteritis (EPEC, ETEC, EHEC, EIEC, EAEC (Parrick*et al.*, 2009)

2.5.2 *Klebsiellapneumoniae*:

Members of the genus*Klebsiella*have a prominent capsule that is responsible for the mucoid appearance of isolated colonies and the enhanced virulence of the organismsin vivo. The most commonly isolated members of this genus are *Klebsiellapneumoniae*and *Klebsiellaoxytoca*, which can cause community- or hospital-acquired primary lobar pneumonia. Pneumonia caused by *Klebsiella*species frequently involves the necrotic destruction of alveolar spaces, formation of cavities, and the production of blood-tinged sputum. These bacteria also cause wound, soft tissue, and urinary tract infections. (Parrick*et al.*, 2009) .

Klebsiella are Gram negative, non-motile, usually capsulated rods. Most *klebsiella*spp are lactose-fermenting, producing mucoid pink colonies on MacConkey agar and yellow mucoid colonies on CLED medium, *K. rhinoscleromatis* is non-lactose fermenting.(cheesbrough , 2006)

2.7.3 *Pseudomonas aeruginosa*:

Pseudomonads are Gram-negative, aerobic, rod-shaped bacteria with widespread occurrence in nature, especially in damp biotopes. The most important species from a medical point of view is *Pseudomonas aeruginosa*. Free O2 is required as a terminal electron acceptor to grow the organism in cultures. The pathogenesis of Pseudomonas infections is complex.

The organism can use its attachment pili to adhere to host cells. The relevant virulence factors are: exotoxin A, exoenzyme S, cytotoxin, various metal proteases, and two types of phospholipase C. Of course, the lipopolysaccharide of the outer membrane also plays an important role in the pathogenesis. Some strains can produce a viscous extracellular slime

layer. These mucoid strains are frequently isolated in material from cystic fibrosis patients.

P. aeruginosa possesses an outer membrane as part of its cell wall. The architecture of this membrane is responsible for the natural resistance of this bacterium to many antibiotics. *P. aeruginosa* can only be grown in culture mediums containing free O2 as a terminal electron acceptor. In nutrient broth, the organism therefore grows at the surface to form a so-called pellicle. Colonies on nutrient agar often have a metallic sheen (*P. aeruginosa*; Latin: aes = metal ore). Given suitable conditions, *P. aeruginosa* can produce two pigments, i.e., both yellow-green fluorescein and blue-green pyocyanin.

Pseudomonas infections occur only in patients with weakened immune, The main infections are pneumonias in cystic fibrosis or in patients on respiratory equipment, infections of burn wounds, postoperative wound infections, chronic pyelonephritis, endocarditis in drug addicts, sepsis, and malignant otitis externa.*P. aeruginosa* frequently causes nosocomial infections. (Fritz*et al*, .2005)

2.5.4 *Proteus*Spp :

Member of *enteriobactericea*,that are lactose negative and motile and produce phenylalanine deaminase. There are several species of *Proteus*, but *Proteus mirabilis* and *Proteus vulgaris* account for the vast majority of clinical isolates in this genus. Both produce urease, and the latter is indole positive. Members of this genus also produce H2S.

These bacteria are capable of swarming motility as they differentiate *Proteus* spp. are common causes of UTIs, occasionally in normal hosts and very commonly in those with indwelling catheters or anatomic or functional abnormalities of the urinary tract. UTIs caused by *Proteus* spp. tend to be more severe than those caused by *E. coli*, with a higher proportion representing pyelonephritis.

Proteus spp. are commonly isolated from the bloodstream, the vast majority secondary to UTI, often associated with urinary catheters. *P. mirabilis* may be second only to *E. coli* as a cause of bacteremia from a urinary source. In addition to UTI, *Proteus* spp. may cause miscellaneous other infections, particularly in hospitalized patients. *proteus* may produce several types of pili, the most important of which, known as MR/P fimbriae, is subject to phase variation as a result of an invertible element by which colonization occur.

Addition to produce a potent urease has also been confirmed to be a virulence factor in contributing to both colonizationand stone formation. Indeed, the enzyme, by hydrolyzing urea to form CO2 and ammonia, alkalinizes the urine, which leads to the precipitation of struvite, formation of calculi, and obstruction of urinary catheters.

The kidney stones serve as foreign bodies in which the bacteria are embedded and from which they emerge to cause recurrent infections. Treatments of infections caused by *P. vulgaris* are generally more resistant than *P. mirabilis.*(Parrick*et al.*, 2009)

2.5.5 *Staphylococcus*:

Genus is gram-positive cocci (0.5 to 1.5μm in diameter) that occur singly and in pairs, tetrads, short chains, and irregular grapelike clusters.

Staphylococci are nonmotile, non–spore forming, and usually catalase positive,and they are often unencapsulated or have a limited capsule .

Most species are facultative anaerobes, discriminate between spp coagulase positive (i.e.,*S.aureus)* and coagulase-negative *staphylococci* (CoNS) (*S.epidermidis&S.saprophyticus*)(Parrick*et al.*, 2009)

2.5.5.1 *Staphylococcus aureus*:

Normal flora on human skin and mucosal surfaces can survive on dry surfaces MRSA now the most common cause of community-acquired skin and soft tissue infections Species characterized by the presence of coagulase, protein A and species-specific ribitolteichoic acid with *N*-acetylglucosamine residues ("polysaccharideA"). Virulence factors include structural components that facilitate adherence to host tissues and avoid phagocytosis and a variety of toxins and hydrolytic enzymes .

Diseases include: toxin-mediated diseases (food poisoning, toxic shock syndrome, scalded skin syndrome), pyogenic diseases (impetigo, folliculitis, furuncles, carbuncles, wound infections), urinary tract infection and other systemic diseases. Hospital- and community-acquired infections with MRSA(Methacillin Resistant *S.aureus*) are a significant worldwide problem(Parrick*et al.*, 2009)

2.5.5.2 *Staphylococcus epidermis* and *Staphylococcus saprophyticus*:

S. epidermidis causing Bacteremia; endocarditis; surgical wounds; urinary tract infections; opportunistic infections of catheters, shunts, prosthetic devices, and peritoneal dialysates . While*S.saprophyticus* Urinary tract infections; opportunistic infections(Parrick*et al.*, 2009).

2.5.6 *Enterococcus* species:

Enterococcus species are Gram positive cocci, occurring in pairs or short chains. They are non-capsulate and the majorities are non-motile.(cheesbrough , 2006).

Enterococci are part of the normal intestinal flora. Although they are capable of producing disease in many settings *Enterococci* cause opportunistic urinary tract infections (UTIs) and occasionally wound and soft tissue infections, biliary tract disease and gastrointestinal disorders. Vascular or peritoneal catheters are often points of entry.

Respiratory tract infections are rare. There is sometimes an associated bacteremia, which can result in the development of endocarditis on previously damaged cardiac valves(James*et al*,. 2004)

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study design

The study was prospective a cross sectional and hospital base study

3.2 Study area

The study was conducted in Al-Faisal hospital, Khartoum state.

3.3 Study duration

This study was conducted during May - August 2015.

3.4 Study population

Diabetic patients with recurrent urinary tract infection

3.5 Sample size

One hundred urine samples (n=100) were collected from diabetic patients.

3.6 Inclusion criteria

Diabetic patients with recurrent urinary tract infection and isolates with multidrugsresistant were included

3.7Exclusion criteria

Non diabetic patient ,diabetic patient without recurrent UTI and sensitive isolates towards drugs

3.8Ethical consideration:

Approval to conduct this study was obtained from Research Ethics Committee of medical laboratory science . After explaining the study and its goal, verbal consent was taken from the study recruits before proceeding with the study and collecting sample

3.9 Data collection tool

Data was collected from hospital records

3.10 Collection and identification of plant material

Adansonia digitia and *Tamarindus indica* fruits were collected from spice dealer, Sudan 2015. The plants were taxonomically identified by taxonomist from Medicinal and Aromatic Plant and Traditional Medicine Research Khartoum, Sudan.

3.11 Preparation of the extracts

Extraction was carried out according to the method described by Sukhdev*et al.*, (2008):

Hundred grams of each sample were grinded using mortar and pestle and extracted with 80 % methanol using soxhelt extractor apparatus. Extraction carried out for about eight hours till the solvent returned colorless. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. Finally the extract allowed to dry in Petri dish and the yield percentage was calculated as followed:

Weight of extract obtained / Weight of plant sample * 100

The yield of the extracts is presented in Table 4.1

3.12 Collection of urine samples

Mid-stream urine samples were collected in universal wide mouth sterile urine containers. Specimen was carried in ice bag during transport to the Microbiology lab. The specimens were inoculated under aseptic condition on Cysteine lysine electrolyte deficient (CLED). The inoculated culture media were incubated aerobically at 37 ˚C for 18-24 hrs and examined for growth.

3.13 Wet preparation

Samples were examined for the presence of pus cells and epithelial cells in urine. Pus cells were counted and were reported by using 40x lens.

3.14 Identification

3.14.1 Colonial morphology

Colonial morphology was the first step in the identification of the clinical isolate depending on size, color, edges and fermentation of lactose in CLED.

3.14.2 Microscopic examination

Fixed and dried smears were prepared from growth. Gram`s stain were applied crystal violet stain for 30- 60 sec, washed with water followed by logo`s iodine for 30-60 sec washed again then decolorized rapidly by alcohol, washed immediately with water and covered with safranin for 2 min then washed and examined microscopically by oil immersion lens (×100).

3.14.3 Biochemical test

Biochemical tests including KIA, indole, urease, citrate, esculin hydrolysis and oxidase test as well as inoculation on differential selected media such as Mannitol salt agar (MSA) were used to distinguish between the clinical isolates.

3.15 Susceptibility test against selected antibiotics

A modified Kirby- Bauer susceptibility testing method was used to assess the sensitivity and resistance patterns of the isolates. On Mueller Hinton agar, a suspension of tested isolate was adjusted with 0.5 % McFarland standard then seeded and antibiotic applied were : Ciprofloxacin (30 mcg), Gentamicin (10mcg), Ceftriaxone (30mcg), Imipenem (10 mcg), Cefoxitin (30mcg), Nalidixic acid (30 mcg), Ceftazidime (30 mcg), Vancomycin (30 mcg) and Amoxicillin (20 mcg)/ Calvunic acid (10mcg) (Cheesbrough , 2006).

Plates were left at room temperature then incubated at 37° C for 18-24 hrs and zones of inhibition were measured in mm, results interpreted according to CLSI chart and recorded (Tables 4.4, 4.5 & 4.6).

For Methicillin Resistant *S.aureus* CLSI recommends incubating isolates being tested against Oxacillin or Cefoxitin at 33-35° C (maximum of 35°C) for a full 24 hours before reading (CDC, 2013).

3.16 Purification and storage of isolates

All isolated Gram negative bacilli were kept in nutrient agar and were used in identification and susceptibility tests. 16% v/v glycerol broth media were used for storing bacteria at -20°C.

3.17 Agar diffusion method

The agar well diffusion using Mueller Hinton Agar No. 2 medium for the assay was done to screen the antimicrobial activity of *Tamarindus indica* and *Adansonia digitata* extracts against selected pathogens. The microorganism was activated by inoculating the strain in the nutrient agar incubated for 18-24 hrs at 37C°, each strain was suspend in sterile normal saline. Then 0.1 ml of inoculums (adjusted according to McFarland standard) was inoculated into the molten Mueller Hinton agar media and after proper homogenization it was poured into sterile petri dishes. Wells was made in the seeded plates by using corck-borer No. 4 size (8 mm). For *Tamarindus indica* 50%, 25%, 12.5% and 6.25 % (w/v) and 100%, 50%, 25 % and 12.5 (w/v) for *Adansonia digitata* were introduced into the wells subsequently and all the plates were incubated for 24 hours at 37 C° (Abdallah, 2014)

Positive control was Chloramphenicol and negative control was methanol, each step was under strict aseptic conditions. Bacterial growth was determined by measuring the diameter of the zone of inhibition (in mm)

3.18 Data Analysis was done descriptive by excel sheet

CHAPTER FOUR
RESULTS

One hundred patients (n=100) were enrolled in the study; identification of clinical isolates was done by conventional methods presented in Table 4.2 &4.3, followed bySusceptibility test with selected done by use of well agar diffusion method .

Selected Antibiotics usedfor *E .coli* isolates were Ciprofloxacin, Gentamicin, Ceftriaxone, Nalidixic Acid andAmoxycalvunic Acid. (Table 4.4) and for*Ps .aeruginosa* isolates were Imipenem, Ciprofloxacin, Gentamicin and Ceftazidime (Table 4.5). *S .aureus* isolates Cefoxitin, Ciprofloxacin, Gentamicin andVancomycin (Table 4.6).

Strains which showed drug resistance were; *E .coli* (54.5%), Methacillin Resistant *S .aureus* (MRSA) (18.1%) and *Ps .Aeruginosa* (18.1%) where subjected to *A. digitata* and *T. indica* extractsin different concentration; (Appendix3). The Screening of antimicrobial activity of extracts was done by agar well diffusion method using corck borer No. 4. Each strain was tested in triplicate and means were presented in Tables 4.7, 4.8 & 4.9 as the diameter zone of inhibition exhibited around the wells containing the antibacterial extracts as well as controls measured in mm. (Fig 4.4 &4.4)

Concentrations that has been applied for *T. indica* were (50, 25, 12.5, and 6.25% (w/v), and (100, 50, 25, 12.5 % (w/v)) for *A. digitata.*

Table 4.1: Yield percent of extracts:

Sample	Weight of sample(g)	Weight of extract(g)	Yield %
Tamarindus indica	100	30.906	30.906
Adansonia digitata	100	31.067	31.067

Table 4.2: Biochemical characteristics of isolated Gram negative bacteria from recurrent urinary tract pathogens

Isolated bacteria	Biochemical tests							%
	Indole	Urease	Citrate	KIA				
				Slope	Butt	Gas	H2S	
E. coli	+ve	- ve	- ve	Y	Y	+ve	- ve	25
K.pneumoniae	- ve	+ve	+ve	Y	Y	- ve	- ve	17
P.areuginosa	- ve	- ve	+ve	R	R	- ve	- ve	6.8
P. vulgaris	+ve	+ve	+ve	R	Y	+ve	+ve	5.8

Table 4.3: Biochemical characteristics of isolated Gram positive bacteria from recurrent urinary tract pathogens

Isolates	Catalase	Manitol fermentation	DNase test	Coagulase test	Esculin hydrolysis	%
E. faecalis	-ve	-ve	-ve	-ve	+ve	6.8
S. aureus	+ve	+ve	+ve	+ve	-ve	20.4
S.epidermidis	+ve	-ve	-ve	-ve	-ve	4

Table 4.4: Antibacterial sensitivity test of *E.coli* strainsagainst selected antibiotics:

Isolates	**CIP.**	**Gn.**	**CXR**	**Nx.**	**AMC.**
E.c SD	20	17	30	23	20
E.c 59	11	R	R	R	R
E.c75	24	11	R	R	R
E.c79	R	10	R	R	R
E.c 85	R	12	15	R	R
E.c 92	R	10	R	R	R
E.c 3	R	13	10	R	R
E.c 83	R	R	20	R	R
E.c 60	R	18	R	R	R
E.c 7	R	10	R	R	R
E.c 9	9	19	R	R	R
E.c 1	12	9	R	R	R
E.c 20	18	R	R	R	R

Key word:

EC:*E.coli;* SD: *E.coli* ATCC 25922

R: Resistant

CIP: Ciprofloxacin (30 mcg), sensitive30-40mm

Gen: Gentamicin (10 mcg), sensitive 19-24mm

CXR: Ceftriaxone (30mcg), sensitive 29-35mm

AMC: Amoxicillin (20 mcg)/ calvunic acid (10mcg). Sensitive 18–24 mm

NX: Nalidixic acid (30 mcg) sensitive 22-28 mm

Table 4.5: Antibacterial sensitivity test of *Ps .aeruginosa* strains against selected antibiotics:

Isolates	CIP	Gen	Imp	Cez
***Ps.a*SD**	23	22	25	22
Ps.a 40	20	12	15	20
Ps.a 57	15	10	22	26
Ps.a 65	0	0	0	25
Ps.a 70	19	0	17	28

Table 4.6: Antibacterial sensitivity test of *S.aureus* strains against selected antibiotics:

Isolates	CIP	Gen	AMC	Cefo	Vn
***S.a*SD**	27	19	29	22	18
S.a 12	19	20	20	0	12
S.a 35	20	10	0	11	13
S.a 38	0	16	16	0	10
S.a 98	22	0	21	12	0

Key words:

S.a: *S. aureus*; SD: *S.aureus*ATCC25923

Ps.a: *Ps .aeruginosa* ATCC27853; SD: *P.aeruginosa* ATCC27853

CIP: Ciprofloxacin (30 mcg), sensitive25-33 mm

Gen: Gentamicin (10 mcg), sensitive 16-21 mm

Cefo: Cefoxitin (30 mcg), sensitive 23-29mm

Vn: Vancomycin (30 mcg), sensitive 17-21 mm

AMC: Amoxixillin(20 mcg)/ calvunic acid (10mcg) . Sensitive 28-36mm

Cez:Ceftazidime(30 mcg) , sensitive 22-29 mm

Imp: Imipenem (10 mcg),sensitive 20-28 mm

Table4.7: Comparison between Antibacterial activity of *A. digitata*&*T. indica*extracts wi
concentrations against standard &clinical isolates of*E.coli*(EC)(zone of inhibition in mm)

Isolates	***Adansonia digitata***				***Tamarindus ind***		
	100%	**50%**	**25%**	**12.5%**	**50%**	**25%**	**12.**
EC ATCC 25922	12.7±0.2	10±0.2	8.7±0.0	R	16.7±0.1	13.7±0.1	11
EC 59	13±0.2	9.7±0.2	R	R	15±0.1	13.3±0.1	9.3
EC 75	13±0.2	10±0.2	6.1±0.0	R	16.7±0.1	13.3±0.1	9.3
EC 79	14±0.2	11±0.2	R	R	17.7±0.1	14±0.1	11
EC 85	13.3±0.2	10±0.2	R	R	12±0.1	13.6±0.1	
EC 92	13.3±0.2	7.3±0.2	R	R	16.3±0.1	13±0.1	10
EC 3	14±0.2	10 ±0.2	R	R	15.3±0.1	13.7±0.1	6.7
EC 83	13.3±0.2	10±0.2	R	R	16.3±0.1	11.7±0.1	8.3
EC 60	16.3±0.2	10±0.2	3.3±0.0	R	18.7±0.1	10.3±0.1	5.7
EC 7	20±0.2	8.3±0.2	R	R	20.7±0.1	17±0.1	12
EC 9	15±0.2	4±0.2	R	R	16.3±0.1	12±0.1	
EC 1	17.7±0.2	14±0.2	R	R	19.3±0.1	14±0.1	12
EC 20	17.7±0.2	7.7±0.2	R	R	17.7±0.1	14±0.1	11

* Note that any diameter ≤ 10 means that there was no antimicrobial activity since the siz
mm

Table 4.8: Comparison between antibacterial activity of*A. digitata*&*T. indica* extracts wit against standard *S. aureus*andclinical isolates ofMethicilin Resistant *S. aureus*(S.a):

Isolates	***A.digitata***					***T.indica***		
	Mean of inhibition Zone (in mm)							
	100%	**50%**	**25%**	**12.5%**		**50%**	**25%**	**1**
S.aATCC 25923	21.7±0.4	16.3±0.3	11.7±0.0	9±0.1		21±0.1	18±0.1	9
S.a 11	23±0.4	19±0.3	7.3±0.0	9±0.1		17±0.1	14.7±0.1	8
S.a 35	21±0.4	15±0.3	10.3±0.0	R		31±0.1	14±0.1	5
S.a 38	20±0.4	15.7±0.3	6.7±0.0	9±0.1		31±0.1	9.3±0.1	7
S.a 98	24±0.4	14.7±0.3	12±0.0	6±0.1		27±0.1	21.7±0.1	1

Key word:S.a: *S. aureus,* R: Resistant

* Note that any diameter ≤ 10 means that there was no antimicrobial activity since the siz mm

Table4.9: Comparison between Antibacterial activity of *A. digitata*&*T. indica* concentrations against standard &clinical isolates of *Ps.Aeruginosa*(Ps.a)

Isolates	***A.digitata***					
	Mean of inhibition zone (in mm)				Mean of inhi	
	100%	**50%**	**25%**	**12.5%**	**50%**	**25%**
Ref - *PA* ATCC27853	19±1.1	18.3±0.05	16.7±0.15	13.3±0.1	20.3±0.0	15.3±
PA. 40	18.3±1.1	18±0.05	13.3±0.15	11±0.1	21±0.0	17.7±
PA 57	15±1.1	14±0.05	10.3±0.15	5.7±0.1	15.3±0.0	12.3±
PA 65	14.7±1.1	15±0.05	13.3±0.15	R	15.6±0.0	13±1
PA 71	19±1.1	18.3±0.05	16.7±0.15	13.3±0.1	20.3±0.0	15.3±

Key word:

PA:*Ps.aeruginosa*, R: Resistant

* Note that any diameter ≤ 10 means that there was no antimicrobial activity since the siz
mm

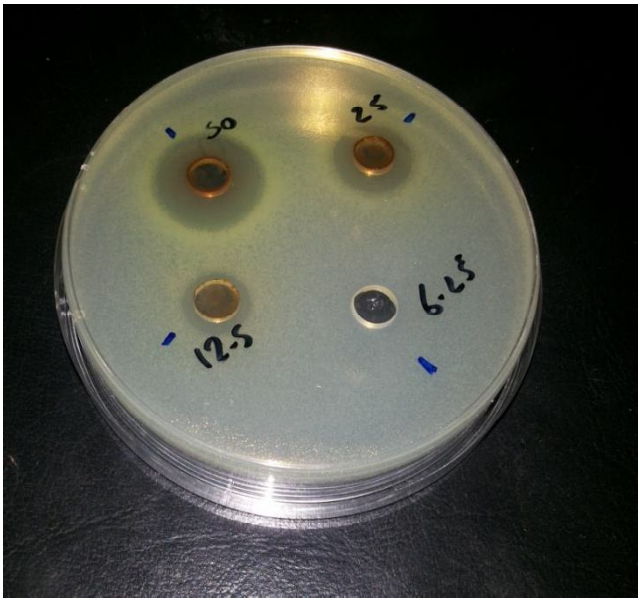


FIG 4.1 Antibacterial activity of *T.indica* against *Ps .aeruginosa*

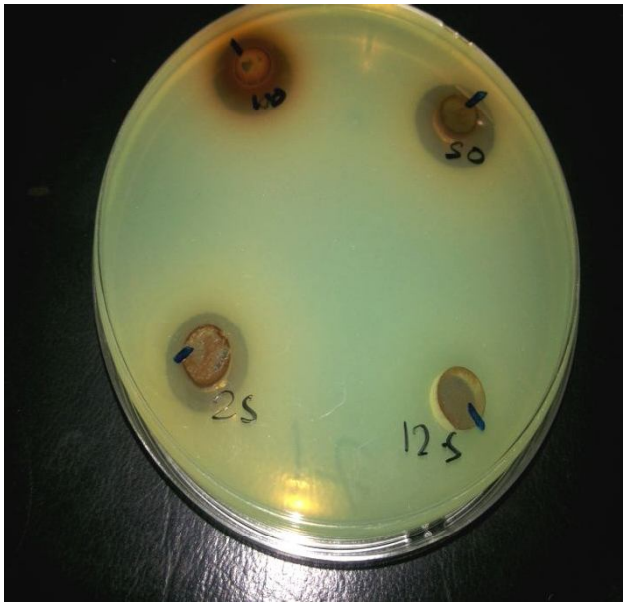


FIG 4.2 Antibacterial activity of*A.digitata* against *Ps.aeruginosa*

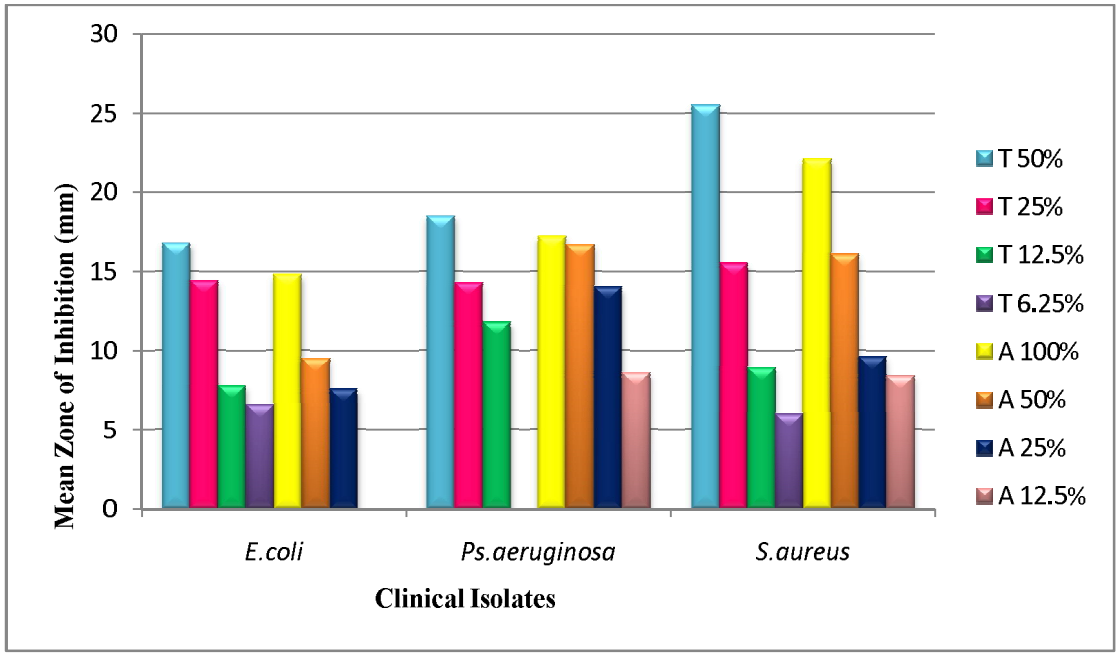


FIG 4.3 Mean diameters of inhibition zones of clinical isolates exposedto various concentrationsof *A. digita* (A) or *T. indica* (T) after 24hrs.

* Note that any diameter ≤ 10 means that there was no antibacterial activity since the size of the corck borer was 8 mm

CHAPTER FIVE
DISCUSSION, CONCLUSION& RECOMMENDATIONS

5.1 Discussion

Due to prescription of antibiotics without sensitivity testing microorganisms develop resistance to many antibiotics. In addition to this many of them are known to have side effects, therefore there is a need to screen local medicinal plants with possible antibacterial properties to find novel alternatives. The objective of this study was to validate Antibacterial activity of *A.digitata* and *T.indica* against selected bacteria isolated from diabetic patients with recurrent urinary tract infection .

One Hundered Urine Samples were collected (n = 100), only (85%) showed bacterial growth, while (5%) were yeast. The isolated bacteria were identified as *E. coli* (25%), *S. aureus* (20.4%), *K. pneumoniae* (17%), *Ps. aeruginosa* (6.8%), *E. feacalis* (5.6%), *P. vulgaris* (5.7%), and *S. saprophyticus* (4.5%). These findings agreed with the results reported by saleem and daniel , (2011) that recoveredisolates were *E.coli*, *E. faecalis*, *Ps. aeruginosa* and *S. aureus;E.coli* was the most common isolate(44.4%).Also *E. coli* was found to be most predominant isolate (54.0%) followed by coagulase negative *Staphylococci* (CoNS) (21.3%) and *Enterococcus* spp. (7.3%)

Resistant strains were *E.coli*(n) =12 (54%) , *S.aureus* (n) = 4(18.1) and *Ps.aeruginosa*n were 4 (18.1%) , similar to Hamdan*et al.*,2015 ; mentioned that *E.coli* were (54%),agreed withShaw *et al.*, 2015 who reported that *E.coli* main organism in multidrug resistantace .

Sharma*et al.*, 2013 mentioned thatthe most predominanat among resitant strains was*E. coli* isolates, 90.8% were MDR strains and most of the isolates had a very high multiple antibiotic resistances (MAR) index.

Isolates that reveal drugs resistance were exposure to methanol extract of both *A.digitata* and *T.indica* in different concentration , Result was varies bettween Species and strains.(Table 4.7 , 4.8 & 4.9)

There is varies in result between strians of *E.coli* isolates in response to different concentration of *T.indica* (50 , 25 , 12.5 , 6.25) % w/v; the zone of their inhibition were ranging from (20.7±0.1 − 12±0.1, 17±0.1 − 10.3±0.1, 12±1.3−0.0) mm respectively ; this agrees with Abdallah , 2014 who reported that *Escherichia coli* ATCC 25922 (11.6±0.6 mm) and complementary toElashi, (2015)showed that*T.indica* extract have an antibacterial effect against *S.mutans* and *E.coli*with a range of 11.41 mm to 7.04mm and 6.88mm to 10.40mm, respectively. Zone of inhibition above 10 mm considered good antibacterial activity (Abdallah, 2014) , also Mukhtar and Gori were stated that zone of inhibition 9-12mm consider as partialy active ; while 13-18mm active and more than 18 mm very active.

E.coli strains expressed susceptibilty towards*A.digitata* this agreed with what reported by Sharma &Rangar, (2015)and Sekup*et al.*,(2013)but diagreed to what mentioned by Oloyede*et al.*, (2010) which they mentioned that no activity was exhibited ; this attributted to different solvents were uesd in extraction and differnent polarity , pH , particle diffusion , various of agriculture system and climate.

For *A.digitata* Concentrationswere used 100, 50 , 25,12.5 w/v and zone of inhibition were ranging from (20±0.2−12.7±0.2,14±0.2−4±0.2, 8.7±0.0−0.0, 0.0) mm respectively.

Ps .aeruginosa strains exhibited equal response to both extracts, in concentartion of 50% for *T.indica* likewise study cnducted by Gumgumjee*et al.*, (2012) which showedAntibacterial effects of *T.indica* Extracts against*E. coli*, *K. pneumoniae*, MRSA, *S. aureus*, *P. aeruginosa* and*B. subtilis*; also agreed Lagnika*et al.*,(2012).

A.digitata showed more activity than *T.indica* which decreased till 6.25 became in active, this agree with what mentioned byYusha'u *et al.*, (2010) were concluded that *A.digitata* is potential source of antibacterial, also complementary to Uchechukwu*et al.*, in 2011 were reported that *T.indica* a good activity against *P.aeruginosa*

Methicillin *S.aureus* strains were displayed highly sensitivity towards*T.indica*, resemble study done by Diplai *et al.*, (2010) that *S.aureus* is the most susceptible more than *P.aeruginosa*and *E.coli* was the least one.*A.digitata*wasdisplayed activityagaint *S.aureus* , is like what mentioned by Sharmaand Rangari, (2015)revealed that *A.digitata*displayed antibacterial activity against*E. coli, Ps. aeruginosa, Salmonella typhi*and *V. cholera.* Utilization of different solvents in extraction and different method to demonestrate antibacterial contribute to varity in results mention in different studies (Yagoub, 2008). In this study methanol extracts of both *A.digitata* and *T.indica* were revealed antibacterial activity towards clinical isolates this attributed to presence of phytochemical compounds. (Doughari, 2006; Yagoub, 2008)

Diplai *etal.*, (2010) subjected *T.indica* extracted with methanol,ethanol and acetone to Gas- Chromotagrohy- Massive-Spectrometery major dominat compounds identified were Furancarboxaldehyde , 2,3-Butanediol m methayl 2- furoate and Hydroxymethylfurfural and 2,2 Diethoxy-5,5-bi-l-pyrroline , Earlier reports about *T.indica*identified other cmpounds and phytochemicals including 2-Furanocarboxaldehyde, 2,3-Butaneidol, methayl 2- furoate and Hydroxymethyl Furfurole and 2,2 Diethoxy-5,5-bi-l-pyrroline (could be responsible for antimicrobial activity.

*A.digitata*Apart from the phytochemicalsfound in *A.digitata* extract, previous studies showedthe presence of an alkaloid namely adansonin (Doughari, 2006) , Also Djeussi*et al.*,2013 mentioned bioactive

compound quercetin-7-O-B-D xylopyranoside , 7-baueren-3-acetatefound in*A. digitata* extract, which thought to be responsible for Theantibacterial activity.

The variation of susceptibility of the tested microorganisms could beattributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts (Khan *et al*., 2009).

In conclusion the results of this study showed that medicinal plants such as *T.indica*and *A.digitata* could be a novel source for theurapautic agents; both extracts have a potiential antibacterial activity that may be due to presnces of various susbstances such as alkaloids, flavoniod, tannins and several aromatic compound.

5.2 Conclusion

In this study the most resistant isolates of Diabetic patients with RUTI were *E.coli* , *P.aeruginosa* and *S.aureus*.Methanolic extract of *T.indica*showed broad spectrum activity compared to the antimicrobial activity of*A.digitata*. *P.aeruginosa* and *S.aureus* strains were more susceptible to *T. indica* and*A.digitata*than *E.coli*.

5.3 Recommendations

1-Urinalysis and midstream urine for culture and sensitivity should be performed with the occurrence of first symptoms in order to establish a correct diagnosis of recurrent urinary tract infection.

2-Examine both extracts on different isolates and reference strains,usingextracts isolated by using different methods and different solvents for the extraction process.

3- It is recommended to determine the minimum inhibitory concentration (MIC) as well as the minimum bactericidal concentration (MBC) and time kill kinetics. More phytochemical analysis to identify active ingredients and testing their antimicrobial properties as well as their pharmacologicalproperties

4- Further *in vivo* studies and clinical trials after isolation and characterization of the bioactive componentsis required to validate these results.

REFERENCES

1. **Abdullah, E.** (2014). *In vitro* antibacterial activities of the crude methanol extract of *Tamarindus indica L* fruit pulp, a native drink from Sudan *Ind. J. Fund. Appl Life Sci.*, **4** (3): pp. 74-78. ISSN: 2231–6345
2. **Addy, R.** (2009). Baobab approved as food ingredient in US retrieved fromhttp://nutraingredientsus.com/content/view/print/2595742009
3. **Ana, L., Flores, M., Jennifer, N. W., Michael, C. & Scott J. H.** (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *J NRMicro,***13**; 269-284.
4. **Anu, Paul Das, M. and Banerjee, A.** (2014) .Extraction of tamarindpulpand its antibacterial activity. *J PSR* .**4** (2):47-49. ISSN: 2249-7412
5. **Barbour, E.K., Al Sharif, M. ,Sagherian ,V. K., Habre, A. N., Talhouk, R. S.** (2004). Screening of selected indigenous plants of Lebanon for antimicrobial acitivity. *J Ethno.*, **93**(1): 1–82
6. **Becker, B**. (1983). The contribution of wild plants to human nutrition in the Ferlo (Northern Senegal). *Agro forestry Systems*; **1**: 257-267.
7. **Bentley R. and Trimen H.** (2004). Medicinal Plants. Vol 1. New Delhi: Asiatic Publishing House
8. **Bhadoriya, S. S., Ganeshpurkar, A., Narwaria, J., Rai, G., and Jain, A. P.** (2011). *Tamarindus indica*: extent of explored potential. *PharmacognRev*; **5**(9): 73-81.
9. **Boyanova, L., Mitov, I.** (2013). Antibiotic Resistance Rates in Causative Agents of Infections in Diabetic Patients. *Expert Rev Anti Inf* ;11(4):4

10. **Buchmann, C., Prehsler, S., Hartl A., and Vogl, C. R.** (2010). The importance of baobab (*Adansonia digitata L.*) in rural West African subsistence-Suggestion of a cautionary approach to international
11. market export of baobab fruits. *JFR;* **2** (3). ISSN 1927-0887 E-ISSN 1927-0895
12. **Burkill, H. M.** (1995). The useful plants of west tropical Africa. Royal Botanic Gardens Kew. vol 2.pp 636. published by Royal Botanic Gardens Kew, United Kingdom
13. **Centers for Disease Control and Prevention (CDC)** (2013). www.cdc.gov/mrsa/lab
14. **Cheesbrough, M.** (2006). District Laboratory Practice in Tropical Countries, second edition. p 65, 67 ,395
15. **Chen, S. L., Jackson, S. L., and Boyko, E. J.** (2009) .Diabetes mellitus and urinary tract infection: epidemiology, pathogenesis and proposed studies in animal models. *J Urol*,**182**(6 Suppl):S51-6.
16. **Chowdhury, M.A.N., Ashrafuzzaman, M., Ali, M.H., Liza, L.N. and Zinnah, K.M.A.,** (2013). Antimicrobial activity of some medicinal plants against multi drug resistant human pathogens. *Adv. Biosci. andBioengi*, **1**(1), p.1.
17. **Coutino-Rodriguez, R., Hernandez-Cruz P. and Gillis-Rios, H.** (2001). Lectins in fruits having gastro-intestinal activity and their participation in the hemagglutinating property of *Escherichia Coli 0157. Arch Med Res.*;**32**:251–9.
18. **Daniyan, S.Y. and Muhammad, H.B.** (2008). Evaluation of the antimicrobial activities and phytochemical properties of extracts of *Tamaridus indica* against some diseases causing bacteria. *Afr. J. Biotech.*, **7**(14).
19. **Dipali, Y. J., Akshaya, K.,Sahooo, J. S., Ghosh , R., Ranveer, C., Aruna , M., Mali, M.** (2010). Phytochemical detection and *in*

*vitro*evaluation of Tamarind fruit pulp for potential anti activity. *Int. J. Trop. Med.* **5**(3):68-72

20. **Djeussi, D.E., Noumedem, J.A., Seukep, J.A., Fankam, A.G., Voukeng, I.K., Tankeo, S.B., Nkuete, A.H. and Kuete, V.,** (2013). Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *J ISCMR*,**13**(1), p.1. 13:164

21. **Doughari, J.H.,** (2006). Antimicrobial activity of Tamarindus indica Linn. *Trop J Pharm Res*, **5**(2), pp.597-603.

22. **Elashi, B.** (2015). The antimicrobial and antifungal efficacy of indigenous plant extracts against *Streptococcus mutans*, *Escherichia coli* and *Candida albicans* .electronic theses university of cape town

23. **El Tahir, B. A., Fadl , K. E. M, and Fadlalmula, A. G. D.** (2010) . Forest biodiversity in Kordofan region, Sudan: effects of climate change, pests, disease and human activities. *J BES.* **11**:34–43.

24. **Foxman, B. (**2002). Epidemiology of urinary tract infections: incidence, morbidity and economic costs. *Am J Med.*, **113** (1A):5S-13S.

25. **Foxman, B**. (2010). the epidemiology of urinary tract infection. *J Nat. Rev. Urol.*;**7**(12):653-660

26. **Fritz, H. K., Kurt A. B., Johannes, E., Rolf M. Z.**(2005) Medical Microbiology. p308 ch 4

27. **Gebauer, J., Kamal, E.S. and Georg, E.** (2002). The potential of underutilized fruit trees in Central Sudan. In Conference on International Agricultural Research for Development. Witzenhausen, Germany.1–6.http://www.agrar.uni-kassel.de/opats/pub/ 2002Gebauer05.

28. **Glew, R. S., Vanderjagt, D.J., Chuang, L.T., Huang, Y.S., Millson, M. and Glew, R. H.** (2005) . Nutrient content of four edible wild plants from West Africa. J *Plant Foods Hum Nutr*, *60*(4), pp.187-193.

29. **Gumgumjee, N.M., Khedr, A. and Hajar, A.S.(** 2012). Antimicrobial activities and chemical properties of *Tamarindus indica* L. leaves extract. *Afr. J. Microbiol. Res.*, 6 (32), pp.6172-6181.

30. **Hamdan, H.Z., Kubbara, E., Adam, A.M., Hassan, O.S., Suliman, S.O. and Adam, I.,** (2015). Urinary tract infections and antimicrobial sensitivity among diabetic patients at Khartoum, Sudan. *J Ann. Clin. Microbiol. Antimicrob.14*(1), p.1.

31. **Hänsel, R., Keller, K., Rimpler, H. and Schneider, G.** (1992). Springer Verlag;.Hagers Hand buch der Pharmzeutischen Praxis.5th ed. Berlin; p. 893

32. **Havinga , R. M., Hartl , A., Putscher , J., Prehsler, S., Buchmann, C., and Vogl C. R. (**2010) .*TamarindusindicaL.* (*Fabaceae*): patterns of use in traditional African *medicine. J Ethnopharmacol*; **127**(3): 573

33. **Ikram, R., Psutka, R., Carter, A. and Priest, P.** (2015). An outbreak of multi-drug resistant Escherichia coli urinary tract infection in an elderly population: a case-control study of risk factors. *BMC Infect.Dis.*, **15**(1), p.1.

34. **Imam, S., Azhar, I., and Hasan M. M.**(2007). Two triterpeneslupanone and lupanol isolated and identified from *Tamarindus indica. PaKJ Med Sci.*;**20**:125–7.

35. **James, J. C., Lawerence, D., Fredrick C. N., James J.P.** (2004). Sherris Medical Microbiology. ch 17 p294

36. **Kamatou, G.P.P., Vermaak, I. and Viljoen, A.M.,** (2011). An updated review of *Adansonia digitata*: A commercially important African tree. *S. Afr. J. Bot.*, **77**(4), pp.908-919.

37. **Khalid, H., Abdalla, W.E., Abdelgadir, H., Opatz, T. and Efferth, T.**, (2012). Gems from traditional north-African medicine: medicinal and aromatic plants from Sudan. *JNat Prod Bioprospect*, **2**(3), pp.92-103..

38. **Johnsson, K.M., Ptaszynska, A., Schmitz, B., Sugg, J., Parikh, S.J. and List, J.F.,** (2013). Urinary tract infections in patients with diabetes treated with dapagliflozin. *J. Diabetes Complicat.*, **27**(5), pp.473-478.

39. **Jose,S.**(2009).Agroforestry for ecosystem services and environmental benefits: an overview. *AgroforestSyst Journal* (**76**)1–10.

40. **Khalid, H., Abdalla, W.E., Abdelgadir, H., Opatz, T. and Efferth, T.**, (2012). Gems from traditional north-African medicine: medicinal and aromatic plants from Sudan. *Nat. prod. Biopro.***2**(3), pp.92-103.

41. **Khan, R., Islam , B., Akram ,M., Shakil,S. , Ahmad ,A., Ali,M. ,Siddiqui , M., and Khan , A.**(2009). Antimicrobial Activity of Five Herbal Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. *J. Mole.* (**14**) 586-597; ISSN 1420-3049

42. **Kothari, V. and Seshadri, S.**(2010). In vitro antibacterial activity in seed extracts of *Manilkarazapota*, *Anonasquamosa* and *Tamarindus indica*. *Biol. Res.*, **43**(2), pp.165-168.

43. **Lagnika, L., Amoussa, M., Adjovi, Y. and Sanni, A.** (2012). Antifungal, antibacterial and antioxidant properties of *Adansonia digitata* and Vitexdoniana from Bénin pharmacopeia. *J. PharmacognosyPhytother.* **4**(4), ISSN 2141-2502, pp. 44-52

44. **Levy, S. B., and Marshall, B.** (2004). Antibacterial resistance world-wide: Causes, challengesand response. *Nat. Med.***10**. p 122-129

45. **Lockett, C.T., Calvert, C.C., Grivetti, and L.E.** (2002). Energy and micronutrient composition of dietary and medicinal wild plants

consumed during drought. Study of rural Fulani, Northeastern Nigeria. *Int. J. Food Sci. Nutr* .(**51**), 195–208

46. **Machado, T.B., Pinto, A.V., Pinto, M.C.F.R., Leal, I.C.R., Silva, M.G. ,Amaral , A.C.F.** (2006) . *Tropical Journal of Pharmaceutical Research*; 5 (2): 597-603

47. **Magrate , M. K., Saifuddin , F. D., Joseph, M. N.,Catherine ,W. L., Fredrick ,M. M.** (2014) .Antimicrobial Activity, Toxicity and Phytochemical Screening of Four Medicinal Plants Traditionally Used in Msambweni District, Kenya Journal of Biology, Agriculture and Healthcare . **4**(28).ISSN 2224-3208

48. **Mukhtar, S. and Ghori, I.** (2012). Antibacterial activity of aqueous and ethanolic extracts of garlic, Cinnamon and turmeric against Escherichia coli atcc 25922 and Bacillus subtilisdsm 3256. *Int. J. Appl. Biol. Pharm. Technol*, **3**(2), pp.131-135.

49. **Nicoletti, J., Kuster, S.P., Sulser, T., Zbinden, R., Ruef, C., Ledergerber, B. and Weber, R.,** (2010). Risk factors for urinary tract infections due to ciprofloxacin-resistant Escherichia coli in a tertiary care urology department in Switzerland. *Swiss Med Wkly*, *140*, p.w13059.

50. **Oloyede, G.K., Onocha, P.A., Soyinka, J., Oguntokun, O. and Thonda, E.** (2010). Phytochemical screening, antimicrobial and antioxidant activities of four Nigerian medicinal plants. *Ann. Biol . Res* , *1*(2), pp.114-120

51. **Osman, M.A.,** (2004). Chemical and nutrient analysis of baobab (Adansonia digitata) fruit and seed protein solubility. *Plant Foods Hum Nutr.***59**(1), pp.29-33.

52. **Parrick, R. M., Ken, S. R. and Micheal, A. P**. (2009). Medical Microbiology 6th edition. p209 ,212

53. **Platt, F. W., and Keating K. N.**(2007) . Differences in physician and patient peun complicated UTI symptom severity: understanding the communication gap**.** *Int J ClinPract*, 61:303-8

54. **Rahul, J., Jain, M.K., Singh, S.P., Kamal, R.K., Naz, A., Gupta, A.K. and Mrityunjay, S.K.** (2015). Adansonia digitata L.(baobab): a review of traditional information and taxonomic description. *Asian Pac J Trop Biomed*, **5**(1), pp.79-84.

55. **Retnam K. and De Britto A.** (2007). Antimicrobial activity of Amedicinal plant Hybanthusenneaspermus (linn) F. *Muell. N. Prod. Rad.* **6(5)**:366-368.

56. **Routhu, N., Rao, B.V., Ramakrishna, G. and Nagaraju, N.** (2015). Antioxidant and antimicrobial activity of *Tamarindus indica L* Leaves. *Int. J. Adv. Res. Sci. Technol. Volume*, *4*(3), pp.333-338.

57. **Saber, M. H., Barai , L., Haq, J. A ., Jilani, M. A., Begum, M. J.**(2010).The pattern of organism causing urinary tract infection in diabetic and non-diabetic patients in Bangladesh. *Bangladesh J. Med. Microbiol.*4 (1), 6–8.

58. **Saleem, M. and Daniel, B. (**2011). Prevalence of urinary tract infection among patients with diabetes in Bangalore city. *Int. J. Emerg Sci.*, **1(**2), p.133.

59. **Santosh, S. B. , Aditya, G. , Jitendra, N. , Gopal, R., and Alok, P. J.**(2011). *Tamarindus indica*: Extent of explored potential.*PharmacognRev*; **5**(9): 73–81.

60. **Seukep, J.A., Fankam, A.G., Djeussi, D.E., Voukeng, I.K., Tankeo, S.B., Noumdem, J.A., Kuete, A.H. and Kuete, V.** (2013). Antibacterial activities of the methanol extracts of seven Cameroonian dietary plants against bacteria expressing MDR phenotypes. *Springerplus*. volume**2**, p.363.

61. **Sharma, A.R., Bhatta, D.R., Shrestha, J. and Banjara, M.R.**(2013). Antimicrobial susceptibility pattern of *Escherichia coli* Isolated from Uninary Tract Infected Patients Attending Bir Hospital. *Nep. J. Sci. Techn.***14** (1), pp.177-184.

62. **Sharma, A., and Rangar ,V**. (2015). Antibacterial, Antifungal and Antitubercular Activity of Methanolic Extracts of *Adansonia digitata. Int. J. Pharm. Biol. Sci*, **10** PP 52-60. Issue 4 Ver. II

63. **Shaw, E., Benito, N., Rodríguez-Baño, J., Padilla, B., Pintado, V., Calbo, E., Pallarés, M., Gozalo, M., Ruiz-Garbajosa, P. and Horcajada, J.P., (**2015). Risk factors for severe sepsis in community-onset bacteraemic urinary tract infection: Impact of antimicrobial resistance in a large hospitalized cohort. *J. Infect.*, **70**(3), pp.247-254.

64. **Soloviev, P., DaoudaNiang, T., Gaye, A. and Totte, A.,** (2004). Variabilité des caractèresphysico-chimiques des fruits de troisespècesligneuses de cueilletterécoltés au Sénégal: *Adansonia digitata*, Balanitesaegyptiacaet*Tamarindus indica. Fruits*, **59**(02), pp.109-119.

65. **Spellberg, B., Bartlett, J.G. and Gilbert, D.N**.(2013). The future of antibiotics and resistance. *N. Engl. J. Med*, 368(4), pp.299-302.

66. **Stapleton, A.** (2002). Urinary tract infections in patients with diabetes. *AmJ Med,***1**, page 113.

67. **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. pp116.

68. **Sundarambal ,M. , Muthusamy , P. , Radha , R., Jerad , S. A.**(2015).A review on *Adansonia digitata* Linn. *J PharmacognPhyto.*; 4(4):12-16

69.**Tariq, M. ,Chaudhary, S. S., Rahman, K. , Hamiduddin,Zaman ,R., and Imtiyaz, S.** (2013) . *Tamaindus indica* An overview *J of Bio SciOpi* · **1**(2) . p128

70.**Uchechukwu, U. N., Grace, E. O., Vincent, N. C., and Anthonym, I. O.**(2011) .Assessment of *Tamarindus indica* Extracts for Antibacterial Activity.*Int J Mole Sci* .**12**(10): 6385–6396.

71.**Ugoh, Sylvanus , C. and Isa, M. H.** (2013). Phytochemical Screening and Antibacterial Activity of the Fruit and Leaf Extracts of *Tamarindus Indica* (Linn.).sciencepub.net/report.(5)**8**

72.**United Nations Conference on Trade and Development (UNCTAD)** (2005). Market brief in the European Union for selected natural ingredients derived from native species. *Adansonia digitata* Baobab.Available http://www.biotrade.org/ResourcesPublications/biotradebrief baobab.

73.**Wagenlehner, F.M., Bassetti, M., Tirán-Saucedo, J. and Naber, K.G.,** (2014). Recurrent Urinary Tract Infection: Antibiotic Resistance And Guidelines Narrative Summary of Selected Presentations given at the OM Pharma/ViforPharma URO-VAXOM® Summit, held in Buenos Aires,Argentina, on 26th–27th April . *EMJ Urol.*;**1**(Suppl 2):3-13

74. **Wallis, T. E**. (2005). Textbook ofPharmacognosy. 5thed. New Delhi: CBS Publishers and distributors;ch 5, p 22-25

75. **World Health Organization.** (2015). Antimicrobial resistance. Fact sheet N°194 http://www.who.int/mediacentre/factsheets/fs194/en

76.**Yagoub, S.** (2008). Antimicrobial activity of *Tamarindus indica* and *Adansonia digitata* extracts against *E. coli* isolated from urine and water specimens. *Res. J. Microbiol.*, **3**:193–197.

77.**Yusha'u, M., Hamza, M.M. and Abdullahi, N.**(2010). Antibacterial activity of *Adansonia digitata* stem bark extracts on some clinical bacterial isolates. *Int. J. Biomed. &Hlth. Sci. Vol*, *6*(3).

Appendix A

Questionnaire

Date / / 2015

1. Gender: Male ( ) female ( )

2. Age ..... years.

3. Type of controlling diabetes mellitus:

Diet based (),Metoforminbased (), Insulin Based ( ), Glimpried

Herbal based (), othermedication ( )

*If Herbal based mention please

4. Duration: .....

5. Co-morbid conditions: Hypertension ( ) Renal problem( ) Other ( )

*If others mention please.....

6. Episodes of UTI During year

Once in year ( ) Twice ( )

Three times ( ) More ( )

7. Antibiotic receive Yes ( ) No ( )

8. Counseling: Clinic ( ) Diabetic Centre ( )

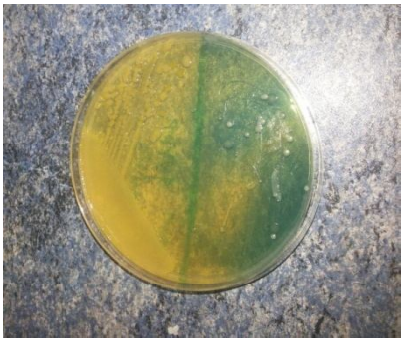
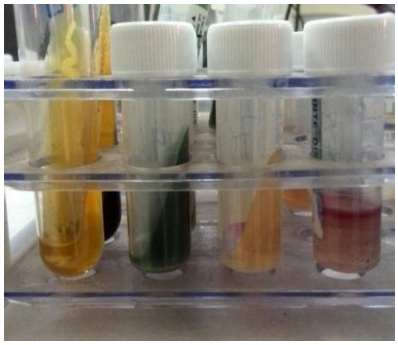
9.Laboratory processing

.....

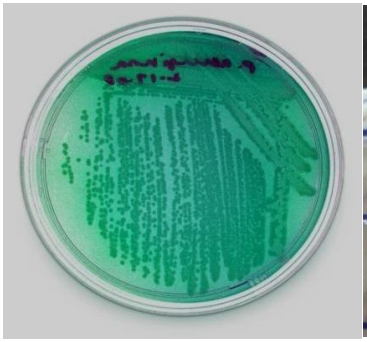
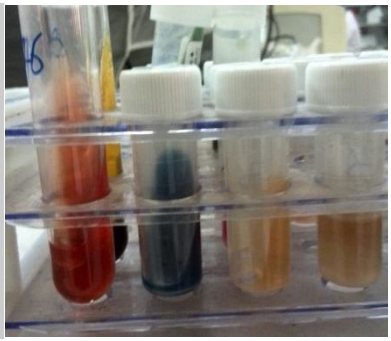
.....

.....

Appendix (1)

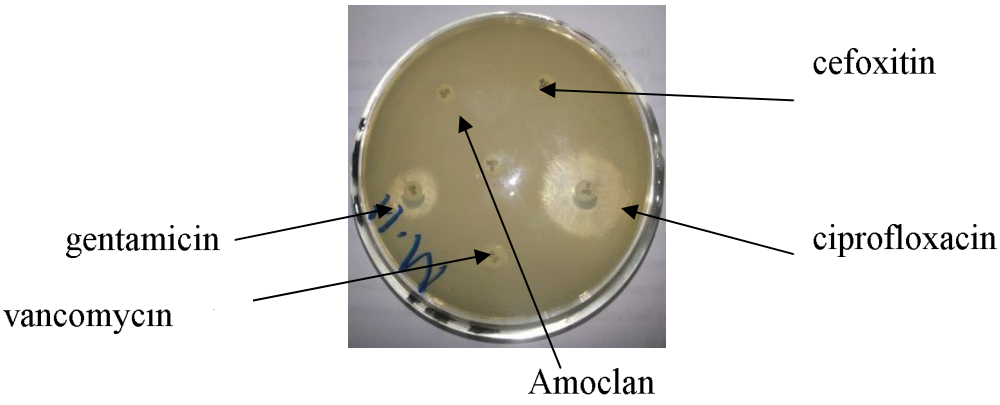
Colour plate (1)showed lactose fermenting Biochemical set

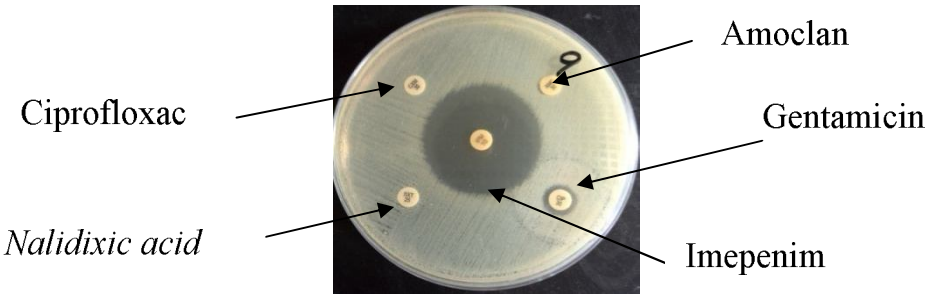
Colour plate(2) *pseudomonas aeruginosa* Biochemical set


Colour plate (3)*S.aureus*showing fermenting mannitol and positive DNasetest

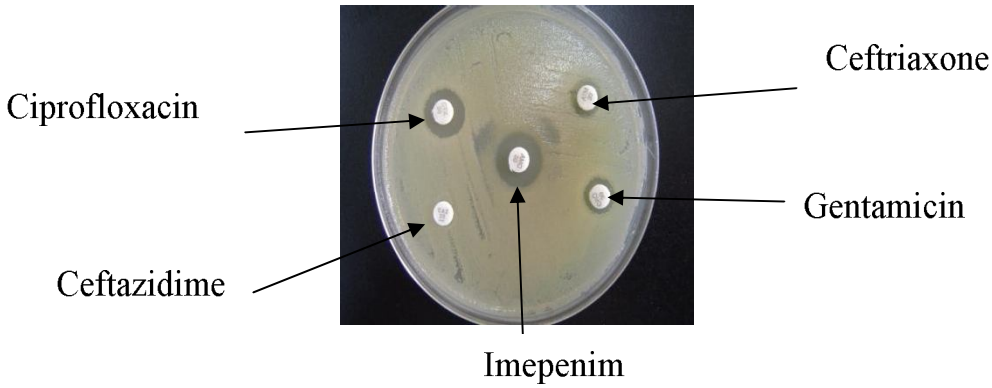
Appendix (2)



Colour plate(4) Methicillin-resistant *staphylococcus aureus*



Colour plate(5) *Escherichia coli*Resistant strains



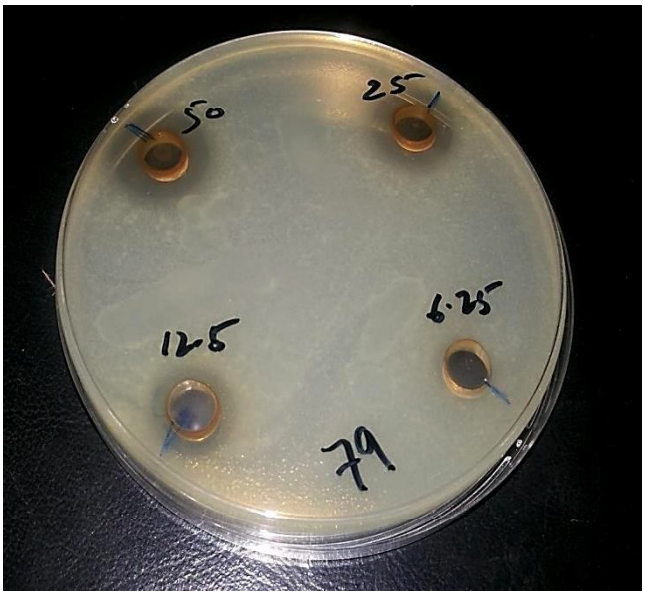
Colour plate(6) *Pseudomonas aeruginosa*Resistant strains

Appendix(3)

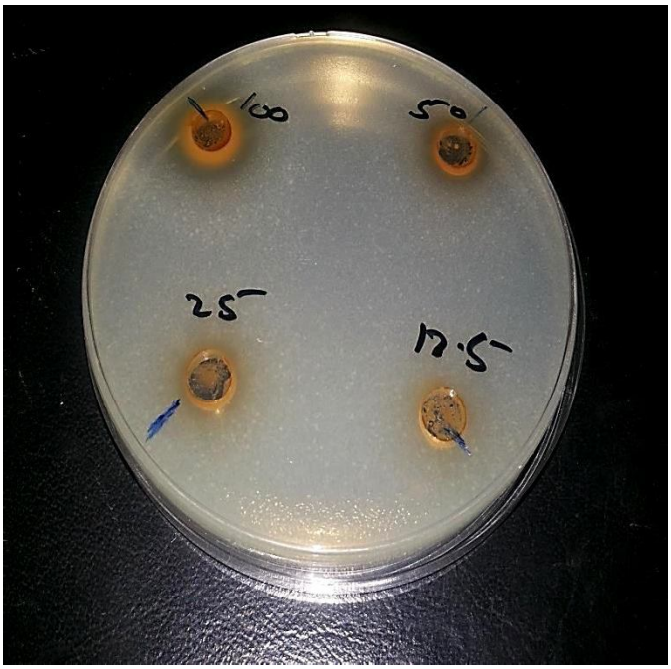
Colour plate (7)*A.digitata* fruit and its extract

Colour plate (8)*T.indica* Extract and its extract

Appendix(4)

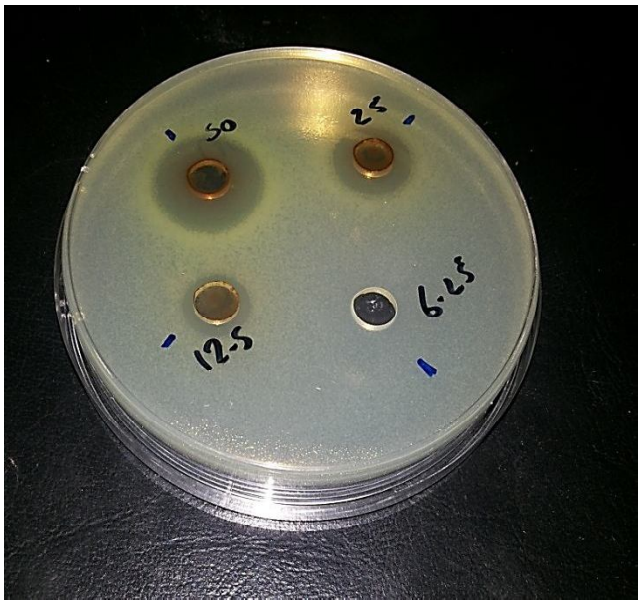


Color plate(9) Antibacterial activity of *T.indica* against *E.coli*



Color plate(10) Antibacterial activity of *A.digitata* against *E.coli*

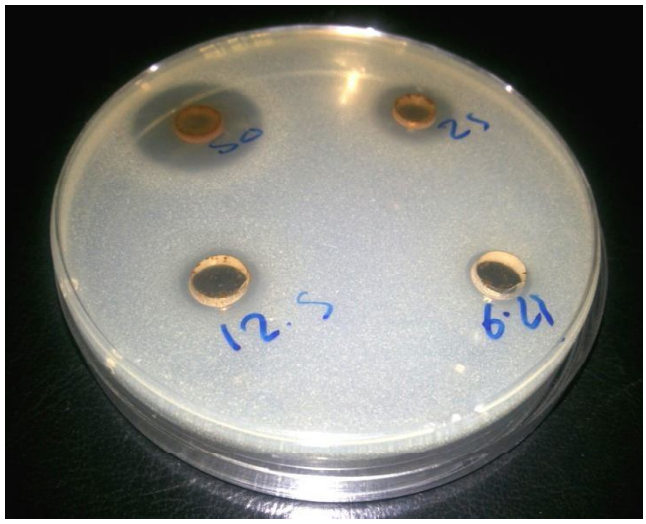
Appendix (5)



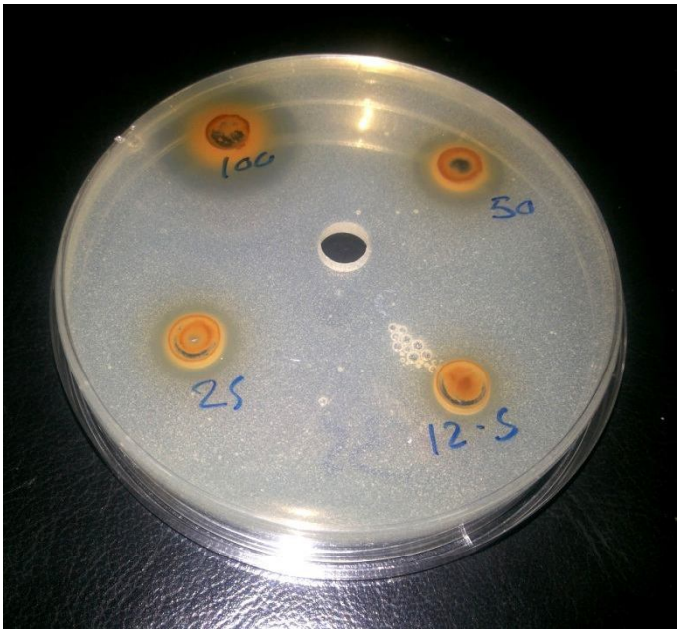
Color plate(11) Antibacterial activity of *T.indica* against *P. aeruginosa*

Color plate(12) Antibacterial activity of*A.digitata* against *P.aeruginosa*

Appendix(6)



Colourplate(13) Antibacterial activity of *T.indica* against *S.aureus*



Colourplate(14) Antibacterial activity of *A.digitata* against *S.aureus*

Appendix (7) Mueller Hinton II Agar

Ingredients	g/L
Beefinfusion	300.0
Caseinacid Hydrolysate	17.50
Starch	1.50
Agar	17.00

preparation:

Suspend 38g of the powder in 1 liter of Distilled water Mix thoroughly. Heat, to completely dissolve the powder. Autoclave at 121^0C for 15 minutes.

Appendix (8): CLED Agar (Cystine-Lactose-Electrolyte-Deficient Agar)

Ingredients	g/L
Enzymatic Digest of Gelatin	4 g
Enzymatic Digest of Casein	4 g
Beef Extract	3 g
Lactose	10 g
L-Cystine	0.128 g
Bromthymol Blue	0.02 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Preparation:

Suspend 36g of the powder in 1 litre of Distilled water, Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121^0C for 15 minutes.

Appendix (9):Nutrient agar

Ingridients g/L

Peptic digest of animal tissue 5.00

Beef extract/yeastextract 3.00

Agar15.00

NaCl5.00

pH is adjusted to neutral (7.4) ± at 25 °C.

Preparation

Suspend 23 g of the powder in 1 litre of Distilledwater . Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121˚C for 15 minutes.

Appendix (10) : Christensen's Urea Agar

Ingredients g/L

Sodium Chloride5.00

Monopotassium Phosphate0.8

Dipotassium Phosphate1.2

Peptone1.00

Dextrose1.00

Phenol Red0.012

Agar 15.0

Final pH 7.4± 0.2 at 25 ° C.

Preparation

Suspend 24 grams in950 ml of distilled water heat boil to dissolve completelyAutoclave at 121 ° C for 15 minutes.thencool to 50° C add Aseptically 40% urea solution and mix well , dispense in strile tube and allow to set at slant position

Appendix (12) Simmon's Citrate Agar

Ingredients g/L	
Sodium Chloride	5.0
Sodium Citrate	2.0
Ammonium Dihydrogen Phosphate	1.0
Dipotassium Phosphate	1.0
Magnesium Sulfate	0.2
Bromothymol Blue	0.08
Agar	15.0

Final pH 7.4± 0.2 at 25 ° C

Preparation

Suspend 24.28 grams in100 ml of distilled water heat boil to dissolve completelyAutoclave at 15 Ibs pressure (121°C)for 15 minutes.thencool to 50° C aseptically dispense in sterile tube and allow to set

Appendix (13) Peptone Water

Ingredients g/L	
Sodium Chloride	5.0
Peptic digest of animal tissue	10.0

Final pH 7.4± 0.2 at 25 ° C

Preparation

Suspend 15 grams in100 ml of distilled water heat boil to dissolve completelyAutoclave at 15 Ibs pressure (121°C)for 15 minutes.thencool aseptically, dispense in sterile tube and allow to set

Appendix (14) :Kligler Iron Agar (KIA)

Ingredients	g/L
Peptic digest of animal tissue	15
Lactose	10.0
Proteose Peptone	10.0
Sodium Chloride	5.0
Beef Extract	3.0
Yeast Extract	3.0
Dextrose	1.0
Sodium Trisulphate	0.3
Ferrous Sulfate	0.2
Phenol Red	0.024
Agar	15.0

Preparation

Suspend 57.52 grams in 1000 ml of distilled water heat boil to dissolve completelyAutoclave at 15 Ibs pressure (121° C)for 15 minutes. then cool aseptically, dispense in sterile tube and allow to set slant position

Appendix (15) :Mannitol Salt Agar (MSA)

Ingredients	g/L
Proteose Peptone	10.0
Sodium Chloride	10.0
Beef Extract	1.0
D-mannitol	10.0
Phenol Red	0.025
Agar	15.0

Preparation

Suspend 111.02 grams in 1000 ml of distilled water heat boil to dissolve completelyAutoclave at 15 Ibs pressure (121° C)for 15 minutes. then cool aseptically, dispense in sterile tube and allow to set slant position

Appendix (16) :DNase Test Agar

Enzymatic Digest of Casein	15 g
Enzymatic Digest of Animal Tissue	5 g
Sodium Chloride	5 g
Deoxyribonucleic Acid	2 g
Agar	15 g

Final pH: 7.3 ± 0.2 at 25°C

Preparation

Suspend 24 g of the medium in 100ml of Distilled water .Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes

Appendix (17) :Antibiotic Disc

CIP	Ciprofloxacin	30 mcg/disc
Gen	Gentamicin	10 mcg/disc
CXR	Ceftriaxone	30mcg/disc
NX	Nalidixic acid	30 mcg /disc
CefoCefoxitin	30 mcg /disc	
VA	Vancomycin	30 mcg/disc
CAZ	Ceftazidime	30 mcg /disc
Imp	Imipenem	10 mcg/disc
AMC	Amoxicillin (20 mcg)/ calvunic acid (10mcg)	/disc

Appendix (18): Autoclave (Medical Instrumention MFG CO,Mumbia)

Appendix (19): Hot air oven (Leader Engineering WidnessCheshire, UK)

Appendix (20): Incubator (TorpePicenardiCCRI, Italy)

Appendix (21): Soxhlet(Duran UK)

Appendix (22): Rotary evaporator (Buchiswitzerland)

Appendix (23): Methanol(Romile EU)