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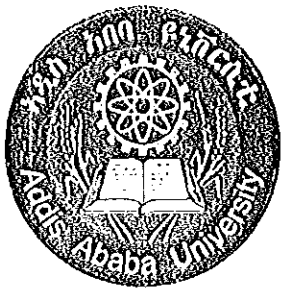

IN VITRO INVESTIGATION OF ANTIMICROBIAL ACTIVITIES OF *ALBIZIA GUMMIFERA* AND *ALBIZIA ANTHELMINTICA* ON MAJOR BACTERIAL UROPATHOGENS ISOLATED FROM ADULT PATIENTS IN ADDIS ABABA

By

Semere Kassaye

July 2006
Addis Ababa

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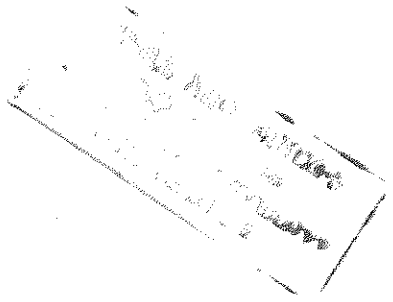

By

Semere Kassaye

A THESIS SUBMITTED TO GRADUATE STUDIES PROGRAM ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN APPLIED MICROBIOLOGY

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ABBREVIATIONS

ATCC: American Type Culture Collection
CD_4 : Cluster Designation 4
CFU: Colony Forming Unit
EHNRI: Ethiopia Health and Nutrition Research Institute
ESβL: Extended Spectrum β-Lactamase
FimH: fimbrial H
HIV: Human Immuno Deficiency Virus
IDSA: Infectious Diseaes Soceity of America
KDa: Kilodalton
mcg: micro gram
MHA: Muller Hinton Agar
MHB: Muller Hinton Broth
ml: milliliter
MSU: Mid stream urine
Mw: Molecular weight
NCCLS: National Committee for Clinical Laboratory Standards
PapG: pyelonephrite association pili G
PNPG: P-nitrophenylglycerol
SMX: Sulfamethoxazole
Spp.: species
TMP: Trimethoprim
TMP-SMX: Trimethoprime - Sulfamethoxazole
WHO: World Health Organization
µg: Microgram
µl: Micro liter

ABSTRACT

A study involving the Collection, Isolation, Identification and Sensitivity screening of major bacterial uropathogens and also the antibacterial activity of two traditionally used plants against the isolates of these uropathogens for various ailments in Ethiopia was conducted at Ethiopian Health and Nutrition Research Institute (EHNRI). The specimens for the isolation of the bacterial uropathogens were collected from adult out-patients with clinically proved symptomatic Urinary tract infections and referred to the EHNRI Reception of the Laboratory Core Service and the Arsho Medical Laboratory by physicians from various Hospitals or Clinics. Out of the total 241 clean catch mid stream urine samples 66 were revealed to have significant bacteriuria. The most frequently isolated species was *E.coli* (77.3%) followed by *Klebsiella spp.* (12.1%), *Pseudomonas spp.* (4.5%), *Proteus spp.* (4.5%) and *Enterobacter spp.* (1.5%). In this study, high level multidrug resistance was observed particularly for commonly prescribed antibiotics: Amoxycillin (69.7%), Ampicillin (64.6%), Tetracycline (53.0%) and Co-trimoxazole (43.9%) using disc diffusion method. The *in vitro* antibacterial activities of 80% methanol crude extracts and their hydro alcoholic solvent fractionates prepared from both seeds of *Albizia gummifera* and *Albizia anthelmintica* were tested for their antibacterial activity against the clinical isolates of *E.coli* (31), *Klebsiella spp.*(8), *Pseudomonas spp.*(3), *Proteus spp.*(3) and also standard reference strains of *E.coli* ATCC,25922 and *P.aeruginosa* ATCC,27853 using agar dilution method. The chloroform fraction didn't exhibit any activity while n-butanol fraction was found to have strong antibacterial effects as compared to the aqueous solubilized fraction which showed antibacterial activity only against *E.coli* and *Klebsiella spp.* at 1000µg/ml. Minimum inhibitory concentration values of n-butanol fraction against the clinical isolates of *E.coli* showed stronger activity than the commonly used broad spectrum antibiotics: Tetracycline and Amoxycillin. Additionally, a better activity was exhibited by this n-butanol fraction against the clinical isolates of *Klebsiella spp.* and *Pseudomonas spp.* than Amoxycillin. Over all, in this study it was found that n-butanol fraction of 80% methanol crude plant extracts of both plants may contain bioactive compounds with therapeutic activity.

Keywords: *Albizia anthelmintica*, *Albizia gummifera*, MIC, Uropathogenic *E.coli*, UTI

1. GENERAL INTRODUCTION

Urinary Tract Infection (UTI) is one of the most important causes of morbidity and mortality among the human population. It can occur at any time in the life of an individual. It is estimated that 150 millions UTIs occur yearly on global basis, resulting in more than 6 billion dollars in direct health care expenditures (Stamm and Norrby, 2001). Acute infections of the urinary tract can be subdivided into two general anatomic related categories: lower tract infection (urethritis and cystitis) and upper tract infection (acute pyelonephritis). Infections at these various sites may occur together or independently and may either be asymptomatic or present as one of the clinical syndromes (Stamm, 2001).

From a microbiological point of view, UTI can be defined as the presence of microorganisms in the urinary tract and most patients with UTI have 'significant bacteriuria'. In most instances, growth of more than 10^5 organisms per milliliter from a properly collected mid stream 'clean-catch;' urine sample indicates infection (Cheesbrough, 2000; Stamm, 2001; Wilson and Gaido, 2004; Ramakrish and Scheid, 2005).

Epidemiologically UTIs are subdivided in to catheter associated (or nosocomial) infections and non-catheter associated (or community-acquired) infections. Infections in either category may be symptomatic or asymptomatic. It has been estimated that symptomatic UTIs results in as many as 7 million visits to out patient clinics, 1 million visits to emergency departments, and 100,000 hospitalizations annually in USA. UTIs have become the most common hospital acquired infections and they are the second most common cause of bacteremia in hospitalized patients (Wilson and Gaido, 2004).

UTIs are not always ascending infections. Bacteria in the blood stream, for instance, patients with pneumonia, can be dispersed and cause a kidney or bladder infection. Such an infection is called a hematogenous UTI (Salyers and Whitt, 1994).

Many different microorganisms can infect the urinary tract, but the most common agents are the Gram-negative bacilli. *Escherichia coli* is the most frequent urinary pathogen isolated from 50%-90% of all uncomplicated UTIs. *E. coli* present in the gastro intestinal tract act as commensals which provide the pool for the initiation of UTIs (Raksha *et al.,* 2003).The *Enterobacteriaceae* are among

the medically most important bacteria groups. A number of genera within the family are human intestinal pathogens (e.g. *Salmonella*, *Shigella*, uropathogenic *E.coli, Yersinia,* etc.). Several others are normal colonizers of the human gastrointestinal tract (e.g. *Escherichia*, *Enterobacter*, *Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans. Among these, *E.coli* that cause UTI are categorized to be Uropathogenic *E.coli* (UPEC).

The bacteria sourced from the feces colonize the perineal region and ascend the urinary tract to the bladder. Bladder infections are more common in females than males by virtue of the shortened urethra. The typical patient with uncomplicated cystitis is a sexually active female who was first colonized in the intestine with a uropathogenic *E. coli* strain. The organisms are propelled into the bladder from the periurethral region during sexual intercourse. With the aid of specific adhesins produced they are able to colonize the bladder.

Other Gram-negative rods, especially *Proteus* and *Klebsiella* and occasionally *Enterobacter,* account for smaller proportion of uncomplicated infections. These organisms and *Pseudomonas aeruginosa* also play a major role in nosocomial, catheter- associated infection (Stamm, 2001). Even though, Gram-positive cocci play a lesser role in UTIs, *Staphylococcus saprophyticus* accounts for 10 to 15% of acute symptomatic UTI in young females. *Enterococci* occasionally cause acute uncomplicated cystitis in women. More commonly, *Enterococci* and *Staphylococcus aureus* infections associate in patients with renal stones or previous instrumentation or surgery (Glauser, 1986; Cheesbrough, 2000; Stamm, 2001).

All these species are equipped with a variety of virulence factors. The best characterized are those from urinary pathogenic *E. coli.* Among the first virulence factors that come into play during establishment of UTI are adhesins (Oleschlaeger *et al.*, 2002; Raz *et al.*, 2004). Other virulence factors of recognized importance in the pathogenesis of UTI include the aerobactin system, hemolysin, K- capsular and resistant factors to serum killing effects.

To assess the prevalence of antimicrobial resistance many national and international surveillance programmes, have already been launched, most of which have focused on comparing resistance problems in general and between countries. In the areas of UTIs, uropathogens have shown steady increase in resistance to several antibiotics over the last decade. *E. coli* and other *Enterobacteriaceae* have become less susceptible to commonly used antibiotics such as Ampicillin, Amoxycillin, Co-

amoxiclav, Sulphonamides, Trimethoprim, Co-trimoxazole and in some geographical areas, Fluoroquinolones (Kalhmeter, 2000).

Reports of different studies conducted in Ethiopia revealed that there is high incidence of resistance to the commonly prescribed antimicrobial agents in the treatment of UTI (Messele Gedebou, 1983; Dawit Wolday and Worku Erge, 1997; Ashenafi Beilu and Lindtjorn, 1999; Moges *et al.*, 2002; Zeleke Wolde Tenssaye, 2002). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use.

Higher plants produce hundreds and thousands of diverse chemical compounds with different biological activities (Hamburger and Hostettmann, 1991). It is believed that these compounds have an important ecological role. They can work as pollinator attractants and as chemical defenses against insects, herbivores and microorganisms (Harborne, 1990; Cowan, 1999; Dawit Abebe *et al.*, 2003). These antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Mitscher *et al.*, 1987; Cowan, 1999).

There are several reports in literature regarding the antimicrobial activity of plant crude extracts and the bioassay guided fractionation of them to yield active principles (Nostro *et al.*, 2000; Palmbo and Semple, 2001; Srinivasan *et al.*, 2001; Aberra Geyid *et al.*, 2005; Edeoga *et al.*, 2005). From an estimated 250,000 higher plants in the world (Wilson, 1988), only 5-15% have been studied for a potential therapeutic value (Balandrin *et al.* 1985; Kinghorn, 1992). A large number remains yet to be investigated.

In Ethiopia like other developing countries, medicinal plants still represent the main therapeutic tool in traditional medicine. The Ethiopian flora offers great possibilities for the discovery of new compounds with antimicrobial activities. It is estimated that 6500 to 7000 species of higher plants occur in Ethiopia of which about 12%are endemic (Gebre Egziabher, 1991). The country is well known for its significant geographical diversity, which favored the formation of different habitat and vegetation zones. Ethiopia is also a home of many languages, cultures and beliefs that in turn have contributed to the high diversity of traditional knowledge and practice of the people, which, among others, include the use of medicinal plants.

more common among elderly men and women, with rates as high as 40 to 50% in accordance with some studies (Stamm, 2001 and Neild, 2005).

In recent perspective study of acute UTIs in young women, the incidence was 0.5-0.7 per year. Recurrent infections become problems in 25-30% of women (Stamm and Norrby, 2001). Some people are more likely to get UTI than others, but about one of every five women will develop at least once a UTI in her lifetime (Rubin *et al.*, 1992). Although men have urinary tract infections, the incidence is much lower. It may be because of the male urethra being longer than the female and also the urethral opening in females is with in the vaginal tract that the female urinary tract is an area more easily colonized by bacteria than the relatively dry skin of the tip of the penis (Salyers and Whitt, 1994).

For many women, sexual intercourse seems to trigger an infection. There is also an association between use of diaphragm and spermicides and increased risk of infection (Salyers and Whitt, 1994 and Neild, 2005). Community acquired UTIs are almost always ascending infections that is, bacteria first infect the urethra (urethritis) and bladder (cystitis). In some cases the infection continues to ascend, and the kidneys are infected (pyelonephritis). Pyelonephritis is a more serious disease than cystitis or urethritis since the kidney is a high-vascularized organ there is more potential for tissue destruction and a blood stream infection (Neild, 2005; Ramakrishna and Scheid, 2005,).

2.2.2. Hospital Acquired Urinary Tract Infections

Hospital acquired UTIs are usually associated with indwelling urinary catheters, approximately 50% of all patients who have an indwelling catheter for more than 5 days will develop a bacterial colonization of the bladder. In many cases, removal of catheter is followed clearance of the bacteria, and a symptomatic infection does not develop (Stamm, 2002; Nicolle, 2001). Other risk factors in hospitalized patients besides catheterization are obstructions or abnormalities of the urinary tract and fecal incontinence. UTIs are common starting points for septicemia in hospitalized patients. In immuno-compromised patients, the progression from simple UTI to systemic infection is even more likely to occur (Shanson, 1989).

Bacteria in the blood stream can seed in the kidneys and cause a kidney or bladder infection. Such an infection is called a hematogenous urinary tract infection. Hematogenous infections almost always

prostatitis, and intra renal and perinephric abscesses). Infections at these various sites may occur together or independently and may either be asymptomatic or present as one of the clinical syndromes (Stamm, 2001).

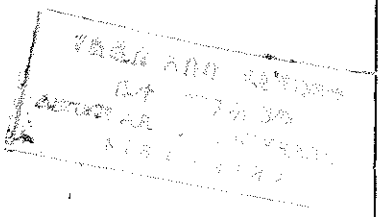
Depending on the factors that trigger the infections, UTIs are also some times further defined as either being uncomplicated or complicated syndromes (Neild, 2005).

Uncomplicated urinary Tract Infection: Cystitis is the most common UTI and is some times referred to as acute uncomplicated UTI. It occurs in lower urinary tract and nearly always in women. In most cases, the infection is brief and acute and only the surface of bladder is infected. Deeper layers of the bladder may be harmed if the infections become persistent (Rubin *et al.*, 1992).

Complicated urinary Tract Infection: occurs in patients with a genitourinary tract that does not function normally, usually due to structural or functional abnormalities. There are wide varieties of diseases and interventions responsible for complicated UTI, these include obstructive lesions, metabolic diseases, instrumentation, foreign bodies, or dysfunctional voiding primarily due to neurological illness (Stamm and Norrby, 2001; Neild, 2005).

2.2 Epidemiology of Urinary Tract Infection

Epidemiologically, UTIs are subdivided into catheter associated (or nosocomial) infections and non-catheter associated (or community acquired) infections. Infections in either category may be symptomatic or asymptomatic.



2.2.1 Community Acquired Urinary Tract Infection

Acute community acquired infections are very common and account for more than 7 million visits to out patient's clinics annually in the United States. These infections occur in 1 to 3% of schoolgirls and then increase markedly in incidence with the on set of sexual activity in adolescence (Stamm, 2001; Neild, 2005). Recurrent UTIs, are problems in 20-25% of women. These are exogenous re-infections (Stamm and Norrby, 2001). Bacteriuria is rare in boys and young men, unless anatomical or functional abnormality of urinary tract is present (Nicolle, 2001). Asymptomatic bacteriuria is

Therefore, the intention of this study was to describe the current prevalence of bacterial uropathogens and their resistance level to various antibiotics in the study sites and then to address the effectiveness of the selected traditionally used medicinal plants against clinical isolates from urine samples along with standard reference strains. In order to achieve the last goal, in the present study two traditionally used plants namely *Albizia gummifera* and *Albizia anthelmintica* were selected. The plants were chosen based on their reported uses in the literature (Thulin, 1989; Dery *et al.*, 1999; Dawit Abebe *et al.*, 2003; Aberra Geyid *et al.*, 2005) and on ethno botanical survey information.

2. LITRUTER REVIEW

The Urinary Tract

The urinary system consists of the kidney, ureters, bladder, and urethra. The key players in the system are the kidneys; a pair of purplish brown bean shaped organs located below the ribs to wards the middle of the back. The kidneys remove liquid waste from the blood in the form of urine, keep a stable balance of salts and other substances in the blood, and produce a hormone that aids the formation of red blood cells. Narrow tubes called ureters carry urine from the kidneys to the bladder, a triangle shaped chamber in the lower abdomen. Urine is stored in the bladder and emptied through the urethra, which is the lowest part of the urinary tract. Urine is normally sterile. It contains fluids, salts, and waste products, but it is free of bacteria, viruses and fungi (Glauser, 1986).

2.1 Urinary Tract Infection

A urinary Tract Infection (UTI) is a condition where one or more structures in the urinary tract has become infected after microorganisms over come its strong natural defenses. UTIs are the most common of all infections and can occur at any time in the life of an individual. Almost 95% of cases of UTIs are caused by bacteria that typically multiply at the opening of urethra and travel up to the bladder (Shanson, 1989).

Acute infection of the urinary tract can be subdivided in to two general anatomical categories. These are lower tract infections (urethritis and cystitis) and upper tract infections (acute pyelonephritis,

involve Gram-positive bacteria like *Staphylococcus aureus*, where as ascending infections are most commonly caused by Gram-negative bacteria. Hematogenous infections are rare cases than ascending infections (Salyers and Whitt, 1994).

2.3. Etiological Agents of Urinary Tract Infection

Many different microorganisms can infect the urinary tract, but by far the most common agents are the Gram-negative bacilli (Table.1). Uropathogenic *Escherichia coli* strains cause approximately 80% of acute uncomplicated UTIs (Stamm, 2002). Other Gram -negative rods, especially *Proteus* and *Klebsiella* and occasionally *Enterobacter*, account for smaller proportion of uncomplicated infections (Stamm, 2001; Ramakrishna and Scheid, 2005).

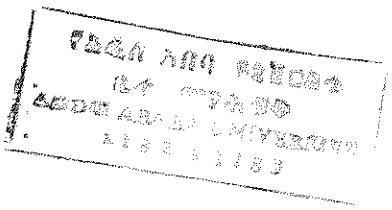
Table.1 Percentage distributions of etiologic agents of urinary tract infections among out-patients and in-patients, by pathogen (Adapted from Wilson and Gaido, 2004)

Pathogen	Out patients %	In patients %
Escherichia coli	53 -72	17.5 - 56.7
Coagulase-negative *Staphylococci*	2 - 7.5	2.1- 12.5
Klebsiella species	6- 12	6.2 - 15.0
Proteus species	4 - 6	3.8 - 8.2
Enterobacter species	0.6 - 5.8	0.9 - 6.5
Morganella morganii	3.1- 4.4	4.7 - 6.0
Citrobacter species	0.1	0.2 - 3
Enterococcus species	1.7- 12	6.5 - 15.8
Staphylococcus aureus	2	1.6 - 3.5
Staphylococcus saprophyticus	0.2 - 2	0.4
Pseudomonas species	0.1 - 4	1.3 - 11
Candida species	...	9.4 - 15.8
Other	3 - 8	1.8 - 26.3

Enterococci and *Pseudomonas aeruginosa* are rare bacterial agents and are most often detected in hospital acquired UTIs. The bacteria that cause acute uncomplicated pyelonephritis are generally the same as those causing cystitis but some evidence indicate that the *E. coli* strains tend to be more virulent (Nicolle, 2001).

A wide range of organisms causes complicated UTIs but *E. coli* is still the most common organism while others have also been detected. These include among the Gram-negative bacteria *Klebsiella spp., Proteus mirabils, Citrobacter spp.*, and others like *Pseudomonas aeruginosa, Enterobacter* and *Serratia species.*

Gram-positive cocci play a lesser role in UTIs. However, *Staphylococcus saprophyticus* accounts for 10 to 15% of acute symptomatic UTIs in sexually active young women (Cheesbrough, 2000). According to Wilson and Gaido (2004) report *Enterococci* have emerged as the second most common cause of nosocomial infections. The causative role of nonbacterial pathogens in UTIs remains poorly defined. However, *Candida species* are noted as the common fungal causative agents and usually found in diabetic patients and those with immuno suppression. *Mycobacterium tuberclosis* is often obtained from patients with chronic fever when there is a pyuria but the routine urine culture is sterile. (Cheesbrough, 2000; Stamm2001).



2.4. Major Bacterial Uropathogens

2.4.1. Uropathogenic *Escherichia coli*

Escherichia coli was first isolated in 1885 by the German bacteriologist, Theodor Esherich, as a normal inhabitant of the intestinal tract Escherich named the organism bacterium coli, the name reflecting the rod shape of the cell (Bacterium means rod shaped) and its intestinal habitat (coli for colon). The genus name Bacterium subsequently was changed to Escherichia in honour of its discoverer (Brock and Madigan, 1991).

E. coli is the head of the large bacterial family, *Enterobacteriaceae,* the enteric bacteria which are facultatively anaerobic Gram -negative rods that live in the intestinal tracts of animals in health and disease. In the late 1970s it was recognized for the first time that *E. coli* strains causing urinary tract

infections typically agglutinate human erythrocytes despite the presence of mannose (Johnson, 1991) and this was mediated mainly by fimbriae. Subsequently an array of virulence factors has been proposed as virulence markers for uropathogenic isolates of *E coli*. It is now recognized that there are a subset of faecal *E. coli* having the above mentioned factors which can colonize periurethral area, enter urinary tract and cause symptomatic disease. These are currently defined as uropathogenic *E. coli* (Raksha *et al*., 2003).

Physiologically, *E. coli* is versatile and well adapted to its characteristic habitats. It can grow in media with glucose, as the sole organic constitute. Wild type *E. coli* has no growth factor requirements, and metabolically it can transform glucose in to all of the macromolecular components that make up the cell. The bacterium can grow in the presence or absence of oxygen. Under anaerobic condition it will grow by means of fermentation, producing characteristic 'mixed acids and gas' as end products. However, it can also grow by means of anaerobic respiration, since it is able to utilize NO_2, NO_3 or fumerate as final electron acceptors for respiratory electron transport processes. In part, this adapts *E. coli* to its intestinal (anaerobic) and its extra intestinal (aerobic or anaerobic) habitats (Brock and Madigan, 1991).

2.4.2. *Klebsiella species*

The genus *Klebsiella* belongs to a member of the family Enterobacteriaceae. The organisms are named after Edwin Klebs, a 19th century German microbiologist. *Klebsiella* species are non-motile, rod-shaped, Gram-negative bacteria with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on Gram stain, and provides resistance against many host defense mechanisms.

Klebsiella species are ubiquitous in nature. They probably have two common habitats, one being the environment, where they are found in surface water, sewage, soil and on plants, and the other being the mucosal surfaces of mammals such as humans, horses, or swine, which they colonize. In this respect, the genus *Klebsiella* is like *Enterobacter* and *Citrobacter* but unlike *Shigella* spp. or *E. coli*, which are common in humans but not in the environment (Bagley *et al*., 1978; Ullmann, 1983; Edberge *et al*.1986). *Klebsiella* species are usually identified and differentiated according to their biochemical reactions.

Klebsiella accounts for 6 to 17% of all nosocomial UTI and shows an even higher incidence in specific groups of patients at risk, e.g., patients with neuropathic bladders or with diabetes mellitus (Lye *et al.*, 1992; Bennet *et al.*1995). Nosocomial *Klebsiella* infections are caused mainly by *Klebsiella pneumoniae*, the medically most important species of the genus. To a much lesser degree, *K. oxytoca* has been isolated from human clinical specimens.

2.4.3. *Pseudomonas aeruginosa*

Pseudomonas is a Gram-negative rod that belongs to the family Pseudomonadaceae. More than half of all clinical isolates produce the blue-green pigment pyocyanin. Often, *Pseudomonas* has a characteristic sweet odor. It is an opportunistic pathogen. *P. aeruginosa* is normally found in soil and water, although it is capable of transiently colonizing the skin and intestinal tract of humans and animals. *P aeruginosa* has been particularly successful in adapting itself to new environments created by human activities. It has few nutritional requirements and can use an impressive array of natural and artificial compounds as carbon and energy sources (Brock and Madigan, 1991).

It can be found growing in hot tubs, mop water, and dilute disinfectant solutions. The normal non-specific defenses of the human body are sufficient to prevent *P. aeruginosa* from causing infections, but serious breaches of these defenses (e.g. burns, immuno suppressive therapy, or wounds), can allow *P. aeruginosa* to invade the body and cause fatal systemic diseases. *P. aeruginosa* also causes urinary tract infections, particularly in people with spinal cord in juries who have permanent indwelling catheters. *P. aeruginosa* strains are notorious for resistance to a wide variety of antibiotics, and number of antibiotics that can be used to treat *P. aeruginosa* infections is small. Resistance to these antibiotics is most likely to encounter in hospital settings, the very setting in which *P. aeruginosa* infections are most likely to be acquired (Salyers and Whitt, 1994).

P.aeruginosa requires oxygen or some other inorganic electron acceptor for growth. It is often described as a non-fermenter because, unlike most other pathogenic bacteria, it is not capable of fermentative metabolism. This doesn't mean that it is an obligate aerobe. *P. aeruginosa* grows well under anaerobic conditions if nitrate is available to serve as an alternative electron acceptor for anaerobic respiration. *Pseudomonas spps.* are remarkably versatile carbon source utilizers (Brock and Madigan, 1991).

2.5. Pathogenesis and Virulence Factors

In the vast majority of UTIs, bacteria gain access to the bladder via the urethra. Ascent of bacteria from the bladder may follow and is probably the pathway for most kidney infections. The vaginal introitus and distal urethra are normally colonized by *Diphtheroids, Streptococcal species, Lactobacilli*, and *Staphylococcal species* but not by enteric Gram-negative bacilli that commonly cause UTIs. The factors that predispose to periurethral colonization with Gram-negative bacilli remain poorly understood, but alteration of the normal vaginal flora by antibiotics, other genital infections or contraceptives (especially spermicide) appears to play an important role (Salyers and Whitt, 1994; Stamm, 2001). Small numbers of periurethral bacteria probably gain entry to the bladder frequently, a process that is facilitated in some cases by urethral massages during sexual inter course. Bladder infection mostly depend on interacting effects of the pathogencity of the strain, the inoculum's size, and both the local and systemic host defense mechanisms (Nicolle, 2001)

A number of defense systems protect the urinary tract against infections causing bacteria, some of which include that: -

- Many bacteria are washed out with normal urination
- Urine itself functions as an antiseptic agent, washing away the potentially harmful bacteria out of the body.
- The ureters are designed to prevent urine from back flowing into the kidney.
- The prostate gland in men secretes infection fighting substances
- The immune systems itself in both sexes continuously fight bacteria and other harmful micro invaders. In addition, the immune system defenses and antibacterial substances in the mucus lining of the bladder eliminate many organisms.
- In normal fertile women, the vagina is colonized by *Lactobacilli*, beneficial microorganisms that maintain a highly acidic environment (low pH) and also produce hydrogen peroxide. These conditions are important to eliminate bacteria and reduce the ability of *E. coli* to adhere to vaginal cells (Schilling *et al.*, 2001).

Gender and sexual activity are important conditions in affecting pathogensis. The short urethra of women favors the ascent of bacteria into the bladder. An important factor predisposing to bacteriuria

in men is urethral obstruction due to prostatic hypertrophy. Homosexuality is also associated with an increased risk of cystitis in men, probably related to rectal intercourse (Stamm, 2001).

Although pregnancy does not increase the rates of asymptomatic bacteriuria, it does increase the risk that it will progress to a full-blown infection. Asymptomatic bacteriuria is found in 4-7% of pregnant women and is associated with premature delivery and low birth weight. Pyelonephritis develops in 30-40% of pregnant women with untreated bacteriuria, compared with 1 to 2% in non-bacteriuric pregnancies (Neild, 2005). This predisposition to upper tract infection during pregnancy results from decrease uretral peristalsis and temporary incompetence of the vesicouretral values. Bladder catheterization during or after delivery causes additional infection (Stamm and Norrby, 2001).

In complicated UTI, there is failure of complete voiding, with persistence of infecting organisms with in the genitourinary tract. Persistence may be due to obstruction with in the urinary tract, with incomplete urine drainage. This also gives a chance for organism to persist in biofilm where a foreign body like catheter is present. There may also be increased access of organisms to the urinary tract (Nicolle, 2001). Immuno suppressed individuals, such as HIV patients, have CD_4 counts of $< 200/\mu l$ and are at increased risk of both bacteriuria and symptomatic UTI (Neild, 2005).

Among the first virulence factors that come in the play during establishment of UTI are adhesins (Latham and Stamm, 1984; Usein *et al.*, 2001; Oleschlaeger *et al.*, 2002: Raz *et al.*, 2004). Bacterial adherence to human uroepithelial cells is increased in *E. coli* that are isolated from symptomatic bacteriuric patients and can be correlated with the presence of hair like structure pili or fimbriae on bacterial cell surface. It is these structures that interact with glycoprotein and glycolipid receptors on host cells. There are two major forms of fimbriae on *E. coli*. These are type I and type P. The adhesin on type I fimbriae is known as FimH whereas on P fimbriae is PapG which are binding to a glycoprotein and a glycosphingolipid receptor of host cells, respectively (Johnson, 1991; Salyer and Whitt 1994; Mulvey *et al.*, 2001; Stamm and Norrby, 2001).

In symptomatic bacteriuiric patients, the strains isolated from the urine are not necessarily the same as those isolated from a random sample of the fecal flora (Johnson, 1991; Raksha *et al.*, 2003). There is an increase in frequency and quantity of virulence factors on *E. coli* organisms cultured from patients with pyelonephritis. One of these factors is K capsular antigen, which appear to be related to increased tissue invasiveness and increased resistance to opsonization and phagocytosis (Glauser,

1986; Schilling *et al.*, 2001). The ability of *E. coli* to gain a foothold in the bladder is greatly facilitated by type I pilus mediated attachment to and invasion of bladder epithelial cell (Mulvey *et al.*, 2001;Schilling *et al.*, 2001).

Capsules are essential to the virulence of *Klebsiella* (Highsmith and Jarvis, 1985). The capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers (Amako *et al.*, 1988). This protects the bacterium from phagocytosis by polymorphonuclear granulocytes, on the one hand, and prevents killing of the bacteria by bactericidal serum factors, on the other (Williams *et al.*, 1983;Williams and Tomas, 1990).

In response to the host defense, pathogenic microorganisms have developed strategies to counter the serum bactericidal effect. Most commensal Gram-negative bacteria are sensitive to the bactericidal effect of human serum, whereas pathogenic strains often exhibit serum resistance properties (Olling, 1987). Thus, clinical isolates of enterobacteria often show resistance to serum (Vosti and Randall, 1990), and the feature "serum resistance" has been correlated with the onset of infection (Olling, 1987) and severity of symptoms (Bjorksten and Kaijser, 1988). Since the main role of the serum bactericidal system is thought to prevent microorganisms from invading and persisting in the blood, even differences in the degree of bacterial serum susceptibility may determine whether a strain is able to infect as well as the length of time it takes the organisms to establish the infection.

Many bacteria attempt to secure their supply of iron in the host by secreting high-affinity, low-molecular-weight iron chelators, called siderophores that are capable of competitively taking up the iron bound to host proteins (Russo *et al.*, 2000). Under iron-deficient conditions, e.g., in the host milieu, enterobacteria synthesize a variety of siderophores, which belong to two different chemical groups, one consisting of the phenolate-type siderophores and the other one consisting of the hydroxamate-type siderophores.

In *E. coli*, the hydroxamate siderophore aerobactin is the most effective of several iron chelating systems employed by enteric bacteria for iron acquisition (Braun *et al.*, 1984). Aerobactin is a small molecular weight chemical (Mw 616) formed from the condensation of two lysine molecules and one citrate. Following secretion by *E.coli* cells, aerobactin extracts Fe^{3+} from host iron binding proteins and is taken up through a 74-KDa outer membrane receptor protein. Strains with the aerobactin

system have a growth advantage in low iron conditions, including in serum and dilute urine (Johnson, 1991).

Other virulence factors of recognized importance in the pathogenesis of UTI include haemolysin. It is a general phenomenon found in many strains of Gram-negative bacteria. Haemolysins can increase the level of available iron in the host via lysis of erthrocytes and eventually release of haemoglobin. There are different haemolysins which have been described in *E.coli*. However, the cytolytic protein toxin secreted by most hemolytic *E.coli* strains is known as alpha hemolysins (Johnson, 1991). Increasing evidence suggested that host genetic factors influence susceptibility to UTI. A maternal history of UTIs is more often found among women who have experienced recurrent UTIs, than among controls. The number and types of receptors on uroepithelial cells to which bacteria may attach are at least in part genetically determined (Stamm, 2001).

2.6. Clinical Presentation

UTI may be asymptomatic. When symptomatic, the clinical manifestation of UTI is basically of two types; the symptoms of lower urinary tract infection (urethritis and cystitis) and the symptoms of upper urinary tract infection (acute pyelonephritis).

Acute uncomplicated UTI is a clinical syndrome characterized by various combinations of dysuria, urgency, frequency gross hematuria, lower back and or abdominal discomfort, and rarely low grade fever (Rubin *et al.*, 1992; Ramakrishna and Scheid, 2005). The urine often becomes grossly cloudy and malodorous, and it is bloody in about 30% of patients with cystitis. White blood cells and bacteria can be detected by examination of unspun urine in most case. However, some women with cystitis have a colony count of $< 10^5$ /ml of urine, and in these instance bacteria can not be seen in a Gram stained preparation. Physical examination generally reveals only tenderness of the suprapubic area (Stamm and Norrby, 2001; Ramakrishna and Scheid, 2005).

In women, the symptoms of odynuria that is, pain or burning during urination and frequency may also be due to vulvitis or vaginitis, both of which are usually accompanied by viginal discharge. It has been suggested that when the complaint of pain or burning is felt to be inside the body and when

vaginal discharge is absent, there is a high probability that the cause is true urethritis due to UTI (Rubin *et al.*, 1992).

It is always difficult to be certain in the presence of cystitis symptoms that the infection is limited to the bladder even in the absence of systemic symptoms such fever. Their mere presence should alert the physician to possible renal infection and or prostatitis or infection elsewhere in the body chronic cystitis with pyuria and sterile urine cultures is highly suggestive of tuberculous infection (Neild, 2005; Ramakrishna and Scheid, 2005). In case of asymptomatic UTI significant bacteriuria is often no associated with symptoms and doesn't require treatment except in pregnant women, in infants and before urological surgery (Gunther *et al.*, 2001).

The syndrome of acute pyelonephritis is less frequently encountered as a manifestation of UTI than the symptoms of urethrocystitis. Acute pyelonephritis is predominantly a disease of young women. When it occurs in men it is associated with a high incidence of obstruction (mainly prostatic hypertrophy), stones, or other urological abnormalities. During pregnancy there is an increased likelihood of developing acute pyelonephritis from UTI (Ramakrishna and Scheid, 2005).

The spectrum of acute pyelopnephritis is wide, ranging from a mild illness to sepsis syndrome. To diagnose acute pyelonephritis, physicians must rely on evidence of UTI from urinalysis or culture, along with signs and symptoms suggesting upper UTI (fever, chills, flank pain, nausea, vomiting, costovertebral angle tenderness). Symptoms that are suggestive of cystitis also may be present. Fever and leukocytosis are of little value in diagnosing acute pyelonephritis in patients who have indwelling bladder catheters, especially when infections are caused by Gram-positive cocci or Candida (Stamm, 2001). In the presence of obstruction (stone, tumor, bladder neck obstruction, enlarged prostate), acute pyelonephritis can be extremely severed and recalcitrant to treatment, and may be progress to renal abscess (Ramakrishna and Scheid, 2005).

2.7. Laboratory Diagnosis of Urinary Tract Infection

The diagnosis of UTI can be made only by finding bacteria in the urine. Urine collection must be performed with great care since voided bladder urine may be contaminated by the resident microbiota of the urethra and vagina. Disinfectants should not be used for cleaning because they may lower the bacterial count if they get into the urine sample (Glauser, 1986). Determination of the number and

types of bacteria in the urine is an extremely important diagnostic procedure (Stamm, 2001; Cheesbrough, 2000). It is necessary to estimate the approximate number of bacteria in the urine because normal specimens may contain small numbers of contaminating organisms usually less than 10^4 cfu per ml of urine.

The most commonly used criterion for defining significant bacteriuria is the presence of $\geq 10^5$ cfu per milliliter of urine (Cheesbrough, 2000; Stamm, 2001). The consensus definition of pyelonephritis established by the IDSA is a urine culture showing at least 10^5 cfu per mm and symptoms compatible with the diagnosis (Ramakrishna and Scheid, 2005). Since the large number of bacteria in the bladder urine is partly due to bacterial multiplication during residence in the bladder cavity, samples of urine from the ureters or renal pelvis may contain $< 10^5$ bacteria per milliliter and yet indicate infection. Similarly, the presence of bacteriuria of any degree in suprapubic aspirates of $\geq 10^2$ bacteria per milliliter of urine obtained by catheterization usually indicates infection (Stamm, 2001).

Several studies have demonstrated the adverse effect of delays in transportation or processing of urine specimens on their quality. Therefore, it is currently recommended that urine specimens be plated with in 2 hours after collection unless specimens have been refrigerated or kept in a preservative (Wilson and Gaido, 2004). Routine urine cultures should be plated using calibrated loops for the semi quantitative method. This method has advantage of providing in formation regarding the number of cfu/ml, as well as providing isolated colonies for identification and susceptibility testing (Cheesbrough 2001). The types of media used for routine cultures should be limited to blood agar and MacConkey agar. In case of out patients urine specimens there is no need of selective media for Gram-positive bacteria since nearly all UTI in out patients are caused by aerobic and facultative Gram-negative bacteria (Wilson and Gaido, 2004).

For urine specimens obtained from hospitalized patients or from patients in whom Gram- positive bacterial infection is suspected, a medium with phenyl ethyl alcohol can be utilized. Because it gives consistent results and allow the growth of both Gram- negative and Gram- positive pathogens cystine lactose electrolyte deficient (CLED) agar is now used by most laboratories to isolate urinary pathogens. Urine cultures should be incubated over night at 35 ^{0}C – 37 ^{0}C in ambient air before being read (Cheesbrough 2000; Wilson and Gaido, 2004). Rapid methods of detection of bacteriuria have been developed as alternatives to standard culture methods. These methods detect bacterial growth by photometry, bioluminescence, or other means and provide results rapidly, usually in 1 to 2 hours.

Compared with urine culture these techniques generally exhibit a sensitivity of 95 to 98% and a negative predictive value of > 99% when bacteriuria is defined as 10^5 cfu/ml (Stamm, 2001).

Microscopy of urine from symptomatic patients can be of great diagnostic value. Microscopic bacteriuria which is best assessed with Gram stained uncentrifuged urine, is found in more than 90% of specimens from patients whose infections are associated with colony counts of at least 10^5/ml, and this finding is very specific. However, bacteria cannot usually be detected microscopically in infections with lower colony counts (10^2 to 10^4/ml). The detection of bacteria by urinary microscopy thus constitutes firm evidence of infection, but the absence of microscopically detectable bacteria doesn't exclude the diagnose. When carefully sought by means of chamber count microscopy, pyuria is a highly sensitive indicator of UTI in symptomatic patients. Pyuria is demonstrated in nearly all acute bacterial UTIs, and its absence calls the diagnosis in to question (Stamm, 2001; Wilson and Gaido, 2004).

Leukocyte esterase method is less sensitive than microscopy in identifying pyuria but is useful alternative where microscopy is not feasible. Here, the tests are based on the hydrolysis of ester substrates by proteins with esteriolytic activity. These proteins react with ester substrates to produce alcohol and acids that then react with other chemical to produce colour change that is proportional to the amount of esterase in the specimen. This test can yield false positive results when the urine is contaminated with bacteria present in vaginal fluid like *Trichomonas species*, which can act as sources of esterase. False negative test results can occur when the urine contains boric acid or excessive amounts of protein (> 500 mg/100ml) or glucose (> 2g /100ml) and also when the patient has received antimicrobial agents, such as Cephalothin, cephalexin, or Tetracycline (Cheesbrough 2000; Wilson and Gaido, 2004.)

Bacteriuria can be detected chemically when bacteria produce nitrite from nitrate. The biochemical reaction that is detected by the nitrite test is associated with the family Enterobacteriaceae. But the usefulness of the test is limited because nitrite production is not associated with urinary tract pathogens such as *Staphylococcus saprophyticus*, *Pseudomonas species*, or *Enterococci* (Cheesbrough, 2001).

The bacterial culture remains an important test in the diagnosis of UTI, not only because it helps to document infection, but also because it is necessary for determination of the identity of the infecting

microorganism and for antimicrobial susceptibility testing. This is particularly true because of the increased incidence of antimicrobial resistance (Wiloson and Gaido, 2004). Therefore, each laboratory should have guidelines by which pathogens are tested for antimicrobial susceptibility. These guidelines should be developed and antimicrobial susceptibility tests should be performed and reported according to the most version of the NCCLS guidelines (NCCLS, 2002).

2.8. Prevention and Treatment

2.8.1. Prevention

Patients with recurrent symptoms may need to experiment with life style changes. The following hygiene tips may be helpful for women at risk for UTIs prevention (Stamm, 2002).

- Clean the genital and urinary areas from front to back with soap and water after each bowel movement
- Avoid tight fitting pants
- Wear cotton crotch under wear and panty hose, changing both at least once a day.
- Takes showers rather than baths
- Avoid bath oils, feminine hygiene sprays, douches and powders.
- Urinate frequently
- Those with residual urine after voiding should practice double micturition.

Behavioral interventions like cessation of spermicide or diaphragm /spermicide use or reduction incoital frequence may be effective but have not been actually studied as interventions (Gunther *et al.*, 2001). When UTI is associated with sexual intercourse, patients may benefit from emptying their bladder afterwards, or from taking a prophylactic dose of antibiotic at the time of intercourse.

Another approach to prevention which is being actively pursued, is the use of a lactobacillus probiotic, namely oral or vaginal application of specifically selected *Lactobacillus* strains with the intent of restoring the normal, *Lactobacillus* dominant flora (Neild, 2005). Cranberry juice has been advocated as a food staff that may prevent UTI by serving as a competitive inhibitor of bacterial attachment to epithelial cells. One controlled trial suggests that regular ingestion of cranberry juice in older women with a symptomatic bacteriuria can reduce the prevalence of a symptomatic bacteriuria, but whether symptomatic infections can be prevented has not been determined (Stamm, 2001; Stamm

and Norrby, 2001). Other studies also support the benefits from the prophylactic effect of cranberry juice was observed in sexual active with recurrent reduction in disease morbidity (Raz *et al.*, 2004).

Women who experience frequent symptomatic UTIs (>3 per year on average) are candidates for long term administration of low dose antibiotics directed at preventing recurrences. Daily or thrice weekly administration of a single dose of TMP-SMZ (80/400mg), TMP alone (100mg), or Nitrofurantoin (50mg) has been particularly effective. Norfloxacin and other fluoroquinolones have also been used for prophylaxis. The same prophylactic regimes can be used after sexual inter course to prevent episodes of symptomatic infection in which UTIs are temporarily related to inter course.

Catheter -induced urinary tract infections are common and preventive measures are extremely important. Catheters should not be used unless absolutely necessary and they should be removed as soon as possible. One study found that catheters impregnated with antibiotics reduced UTI rates during the two weeks they were in place after surgery. Catheter coatings, such as silver nitrate and other substances, are being tested and are showing some benefits, but the problem is still not resolved (Stamm and Norrby, 2001).

It is vital that the integrity of the closed drainage system is maintained at all times. Great care is necessary to avoid contamination of the hands of hospital staff by urine when drainage bags are emptied or changed, and when the bladder is irrigated or samples are taken for investigation. If a catheter is required for long periods, it is best to use it intermittently if possible as opposed to an indwelling catheter it is recommended that replacing it every two weeks and irrigating the bladder with antibiotics between replacements reduce the risk of infection (Shanson, 1989; Stamm, 2001; Stamm and Norrby, 2001).

Extensive research studies demonstrate that control of biofilm formation and inhibition of swarming by *P. mirabilis* may play an important role for prevention of virulence factors. PNPG is a potent anti-swarming agent that could inhibit swarming pattern. It is found that PNPG also inhibits the expression of virulence factors such as protease, urease, hemolysin and flagellum in *P. mirabilis*. In the presence of PNPG the invasion of human uroepithelial cells by *P. mirabilis* is also dramatically reduced. It suggests that PNPG could be used as an active agent against the effects of *P. mirabilis* infections (Liaw *et al.*, 2002)

2.8.2. Treatment

The anatomic location of UTI greatly influences the success or failure of a therapeutic regimen. Bladder bacteriuria (cystitis) can usually be eliminated with nearly any antimicrobial agent to whom the infecting strain is sensitive. It was demonstrated that a single dose of 500mg of intramuscular kanamycin eliminated bladder bacteriuria in most cases. However, a single dose therapy fails in the majority of upper tract infection, and even a 7 day course is unsuccessful in many instances (Stamm, 2001).

A quantitative urine culture, a Gram stain, or an alternative diagnostic test should be performed to confirm infection before treatment is begun, except in acute uncomplicated cystitis in women. When culture results become available, antimicrobial sensitivity testing should be performed to direct therapy (Stamm and Norrby, 2001; Stamm, 2002; Nicolle, 2003). In a study of women with acute uncomplicated pyelonephritis, it was clearly demonstrated that patients who were infected with TMP-SMX resistant strains and treated with TMP-SMX achieved only < 50% cure, compared with > 90% cure in those who received TMP-SMX against TMP-SMX sensitive strains. A three-day course of therapy with TMP-SMX, TMP, Norfloxacin, Ciprofloxacin, or Ofloxacin appears to preserve the low rate of side effects of single-dose therapy while improving efficacy, Thus, 3-day regimens are currently preferred for treating acute cystitis. Neither single dose nor 3 -day therapies should be used for women with symptoms or signs of pyelonephritis, urologic abnormalities, or stones, or previous infections due to antibiotic resistant organisms.

Males with UTI often have urologic abnormalities or prostatic involvement and hence are not candidates for single dose or 3 -day therapy. For empirical therapy, they should generally receive a 7 to 14 day course of a fluroquinolones. Treatment selection in case of women with acute urethritis depends on the etiologic agent involved. In chlamydial infection, azithromycin (1 g in a single oral dose) or doxycycline (100mg orally bid for 7 days) should be used (Rubin et *al.,* 1992; Ramakrishna and Scheid, 2005).

Although the optimal route and duration of therapy have not been established, a 7 to 14 day course of fluoroquinolone, an aminoglycoside, or a third generation cephalosporin is usually adequate in women with acute uncomplicated pyelonephritis but with out accompanying clinical evidence of calculi or urologic disease. Neither Ampicillin nor TMP-SMZ should be used as initial therapy

because > 25% of strains of *E. coli* causing pyelonephritis are now resistant to these drugs *in vitro* (Stamm, 2001;Gupta, 2002).

Complicated UTIs are typically due to hospital-acquired bacteria; including *E.coli, Klebsiella, Proteus, Serratia, Pseudomonas, Enteroccoi*, and *Staphylococci*. Many of the infecting strains are antibiotic resistant. Empirical antibiotic therapy ideally provides broad-spectrum coverage against these pathogens. In patients with minimal or mild symptoms oral therapy with a Fluoroquinolone, such as Ciprofloxacin or Ofloxacin, can be administered until culture results and antibiotics sensitivities are known (Blondeau, 2004).

In patients with sever illness, including acute pyelonephritis or suspected urosepsis, hospitalization and parenteral therapy should be under taken. Commonly used empirical regimens include Imipenem alone, a Penicillin or Cephalosporin plus an Aminoglycoside, and Ceftriaxone or Ceftazidime, therapy should generally be administered for 10 to 21 days, with the exact duration depending on the severity of the infection and the susceptibility of the infecting strain. Following 2 to 4 weeks after cessation of therapy, cultures should be performed in order to demonstrate and confirme cure (Stamm, 2002; Nicolle, 2003).

In pregnancy, acute cystitis can be managed with 7 days of treatment with Amoxycillin, Nitrofurantoin, or a cephalosporin. Acute pyelonephritis in pregnancy should be managed with hospitalization and parenteral antibiotic therapy, generally with a cephalosporin or extended spectrum penicillin. Continuous low dose prophylaxis with Nitrofurantoin should be given to women who have recurrent infections during pregnancy. In all case after treatment, culture is a mandatory test to ensure cure, and culture should be repeated monthly there after until deliver (Nicolle, 2003; Blondeau, 2004).

2.9. Incidence of Antibiotic Resistance in Urinary Tract Infection

Numerous antimicrobial agents introduced over the last 50 years have contributed significantly to the control of infection. However, development of resistance of strains against such agents, as a result of the wide and increasing use of these agents in medicine, veterinary practice and farming has become one of the major concerns for public health (Kalhmeter, 2000; Gullemot *et al.*, 2001; Gupta, 2002). In most parts of the United States, more than one quarter of *E.coli* strains causing acute cystitis are

resistant to Amoxycillin, Sulfa drugs, and Cephalexin, and resistance to TMP and TMP-SMZ is now approaching these levels as well (Gupta, 2003).

Most infections caused by ESβL producing *E. coli* or *Klebsiella pneumonia* had mostly been described as nosocomially acquired, but now these organisms might be an emerging problem among out-patients in different countries. For instance, in a recent nation wide study of ESβL producing organisms in Spain, 93% of ESβL producing *K. pneumonia* strains were isolated from in-patients, while 51% of ESβL producing *E. coli* Strains were isolated from out-patients (Bano *et al.*, 2004).

A recent study from Wales reports consistently higher figures of resistance for 'coliforms' Ampicillin 53.2%, Co-amoxiclav 8.4%, TMP 26.3% and for Ciprofloxacin 2.2% (Howard, *et al.*, 2001). According to Stamm and Norrby (2001) reports of studies clearly demonstrated increasing emergence of antibiotic resistance among srtains of uropathogens causing both community and nosocomially acquired UTIs. Even in women with acute uncomplicated UTI, increasing resistance to Ampicillin (30%-40%), Cephalothin (20%-30%), and TMP-SMX (15%-20%) has been demonstrated in causative *E. coli.*

The comparative analysis of a recent French study to survey in 16 European countries shows broadly corresponding figures for resistance in *E. coli* to Ampicillin (Amoxycillin) (41.3% versus 27.6%), Trimethoprim/ Sulfamethoxazole (21.8% versus 15.1%), Naldixic acid (8.1% versus 3.5%) and Fosfomycin (0.9% versus 1.0%), but considerably lower figures for resistance to Co-amoxiclav (36.7% versus 1.5%)(Kalhmeter, 2003).

Additionally, the study in Europe clearly shows that *E. coli* is now resistant to Ampicillin in > 40% of cases in Spain, Portugal, Ireland and Luxembourg, and to SMX in > 40% in Ireland, Portugal and Spain. Similarly, resistance to TMP and TMP-SMX was observed in > 20% of cases in Germany, Ireland, Portugal and Spain. These results indicate that it is time to seriously reconsider the empirical use of these antibiotics in many countries. It is also necessary to give it time to seriously investigate at which level of resistance the out come of therapy with these antibiotics is influenced, or to develop clear strategies to counteract further resistance development against these drugs.

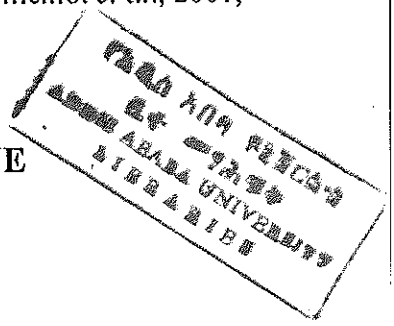
In the retrospective analysis study of 324 positive urine samples in Kashmir medical college it was revealed that 90.12% of the isolates were *E coli* followed by *Klebsiella* (7.72%) and Staphylococcus (1.24%). Significantly 43.57% of the *E.coli* isolates exhibited resistance to the commonly used antibiotics. Similarly, the most effective *in vitro* agents were found to be Amikacin followed by Gentamicin among the injectable and Ciprofloxacin among the orally administered drugs (Kadri *et al.*, 2002).

In Africa, the situation is worrisome. According to a prospective study in Fann University Teaching Hospital in Dakar, the strains of *E. coli* present the leading causative agent in UTI and also show more resistant profile to β-lactams (70.2%) (Sow *et al.*, 2000) than other pathogens. Comparing the studies between Karolinska Hospital (KH) Stockholm, Sweden and Tikur Anbessa Hospital (TAH) Addis Ababa, Ethiopia for *E.coli* isolates from in-patients with UTI it was revealed that a high level of antimicrobial resistance of bacteria has been detected at TAH and also the strains of *E.coli* isolated were considerably more resistant to all seven antibiotics tested. The level of multi resistance was 63% at TAH while it was only 7% at KH (Ringertz, *et al.*, 1990).

According to Messele (1983), a study on etiological agents and their antibiotics resistance patterns indicated that more than 85% of isolated pathogens were resistant to at least one antibiotic. Significant high frequencies of resistance to Ampicillin, carbenicillin, Chloramphenicol, Tetracycline and TMP-SMX were also observed at Tikur Anbessa Hospital by Dawit Wolday and Worku Erge (1997) in their retrospective survey. In other region of Ethiopia, antimicrobial resistance pattern of bacterial isolates in some hospital settings like those at Jimma Hospital, Yirgalem Hospital and Kaffa Regional Public Health Laboratory were recorded (Zeleke Wolde Tenssaye, 1991; Zerusenay Desta and Tefera Abula, 1998; Ashenafi Belihu and Lindtjorn, 1999).

Similarly, the rate of multiple drug resistance was very high in the study carried out at Gondar College of Medical Science Hospital. Isolates tested against ten antibiotics showing more than 68% being resistant to two or more antimicrobials. In their study resistance was high among patient who had a history of catheterization (Moges *et al.*, 2002). This shows that the clinicians are left with only few alternative drugs for UTIs' treatment, even for the remaining chemotherapy, emergence of increasing antimicrobial resistant strains may eventually limit its efficacy. Thus, the need of inventing classes of antibiotics with new mechanisms of action that are not subject to cross resistance with

molecules already in use, is demanding to be given attention in research areas (Guillemot *et al.*, 2001; Kadri *et al.*, 2002).



3. APPLICATION OF TRADITIONAL MEDICINE

3.1. Traditional medicine

According to Akerele (1984), traditional medicine is defined as the sum total knowledge and practice whether they are explained or not. It is used in preventing diseases and elimination of physical, mental, or social imbalance and relying exclusively on practical experiences and observations handed down from generation to generation whether orally or in written form. Historical background suggests that the use of traditional medicine for the treatment of diseases in all countries, whether developed or developing, were considered ancient ideas and indeed even the Bible offer description of 30 healing plants (Cowan, 1999). The World Health Organization estimates that 80% of the population living in the rural areas of many developing countries uses traditional medicine to satisfy their primary health care demands (Bannerman, 1983; Akerele, 1993). An integration of traditional medicinal with modern medicine has been also practiced in countries such as Egypt, Ghana, India, China, Sudan, Serilanka where it is considered as the major health care (Cowan, 1999).

The current demand for herbal remedies in both developed and developing countries is increasing. In developed countries this may be partly due to dissatisfaction with the conventional medicines while with the developing countries this is due to lack of medical doctors, shortages of pharmaceutical products and their unaffordable prices (Tsige Gebre-mariam and Kaleab Asres, 2001). In the USA, it was estimated that 42.5 million visits were made to herbalists in 1990, contrasting with the 388 million actual visits made to primary health care physicians (Eisenberg *et al.*, 1993). Over the counter sales of herbal medicines in the USA and Canada during 1990 reached USD 860 million with an annual growth rate of 15% (Zlhang, 1996).

Traditional medicine in China are reported to account 30-50% of medicines consumed, and the total sales of their herbal medicines amounted to USD 2.5 billion in 1993.With in China, the traditional systems of health care are incorporated in to the formal components of national health care (Bodeker, 1994). In India, there are more than 250,000 registered traditional medicine practitioners, the majority

having received training in degree granting colleges (Bajaj and Williams, 1995). The majority of traditional medicines used in developing countries have not been evaluated for quality, safety, and efficacy to the same standards as those in developed countries. Nevertheless, there are some remarkable claims made for their effectiveness and some traditional medicines have been subjected to 'Western scrutiny' (Anon, 1992).

In Ethiopia, modern health coverage is estimated to be 45%. Drugs are in short supply, and are in accessible and unaffordable to the vast majority of the population .The provision of essential drugs, their equitable distribution and rational use are still serious problems. As a result, about 80% of the population, mainly those in the rural areas rely on traditional medicine for the treatment of both humans and veterinary disease (Tsige Gebre-mariam and Kaleab Asres, 2001). According to Kaba's (1996) reports Ethiopia is one of the six countries of the world where 60% of indigenous plants have healing potential but only few scientific studies have been carried out on indigenous medicine.

The study of traditional cure is clearly of major importance. Such treatment is culturally preferred by large sections of the population, and its development on modern scientific line, is desirable (Pankhurst, 2001) for the following reasons:

1. To understand the true character of Ethiopian's medical history.
2. To seek useful cures for Ethiopia, as well as for the world at large, as has resulted from the study of traditional medicine in other countries;
3. To foster nation dignity, and self reliance, by recognizing the value of traditional local and national knowledge
4. To develop local initiative and industry, thus generating rural wealth, while conserving foreign exchange, this is often spent unnecessarily on the import of foreign drugs.
5. To provide convenient access to medicines more easily available to and acceptable by, rural communities, which would many instances be able to find, or manufacture, their own medicaments.

Despite the wide utilization of medicinal plants in the country, a few research has been conducted to evaluate the crude extracts of traditionally used medicinal plants. Nowadays, because of steady increase of multidrug resistant strains of bacteria in disease like UTI, there is a high demand from a research area to find out an alternative solution against drug resistant bacteria. In view of these facts two traditionally used medicinal plants were investigated for

their possible efficacy on major bacterial uropathogens. The following points offer a brief description about the two plants employed in our investigation.

3.1.1. *Albizia gummifera* (J.F. Gmel) C.A.Sm.

Family: Fabaceae or (Leguminosae).

Vernacular names: Amharic - Sesa, Oromifa - Ambabesa,

It is a deciduous tree preferring forest margins and open forest throughout mountainous region. It is also medium or large tree that occurs mainly in Eastern tropical Africa and also in Cameroon, Nigeria, Madagascar and South Africa at altitudes ranging from 1000 to 2300 m above Sea level. In Ethiopia, this plant is distributed up land riverine forest, 1700-2400m above Sea level in Gojam, Wollega, Illubabur and Kefa (Thulin, 1989). It grows poorly in higher altitudes colonizing woodlands. Means of propagation is directly sowing of seeds or sowing the pod a tree.

Importance of the plant: The wood is medium or large tree fairly strong and does not wrap. It is also used for making beehives, water troughs and boats moreover; *A. gummifera* is a nitrogen fixing tree and can be used for soil stabilization. Its leaves form good mulch and are a good shade tree. It is also common coffee shade. The root system of the plant holds soil and prevents gulley erosion. It has also been reported that *A.gummifera* is being used as traditional medicine for gonorrhea, rectal prolapses, malaria, skin infection (bacterial or fungal), pneumonia, eye diseases, amoebiasis, syphilis, and wound infection in Ethiopia (Dawit Abebe *et al.*, 2003; Aberra Geyid *et al.*, 2005).

3.1.2. *Albizia anthelmintica* (A.Rich)

Family: Fabaceae or Leguminosae

Vernacular names: Amharic - Shina, Somali - Rahydeb, Tigrigna - Bsnna

It is shrub or small tree, deciduous bark smooth, grey to brown young branchets mostly glubrous. Leaves are with 2 to 4 pairs of pinnae, each bearing 2 to 4 pairs of pinnate opposite leaflets. Leaflets obovate to almost circular, up to 3.6 x 3 cm. Flowers usually on leafless twigs, pedicels 0.5-5.5 mm long. Calyx pale greenish, 3-5 mm long. Corolla pale green 6-12 mm long, glabrous, staminal filaments white, about 1.5-2 cm long. Fruit a pod, 7-18 cm long, 1.5-2.9 cm wide, straw colored, papery and pointed. Seeds round and flattened, 6-8 per pod, 9-13 mm in diameter (Thulin, 1989 and Dery *et al.*, 1999).

In Ethiopia, the plant is distributed bush land, woodland and dry scrub 500-1350m above Sea level Tigray, Harerge, Kefa and Gamugofa. It is also found in the Sudan and southwards to Transvaal and Natal (Thulin, 1989).

Importance of the plant: The timber is useful in construction and making furniture. This tree is known to root deeply and has an important role in soil protection and also intercropping potential. *A. anthelmintica* is a shade tree. The species nodulates and is nitrogen fixing. It has also been reported that the plant is being used as traditional medicine for tapeworm in Ethiopia. Avermifuge and taenicide is extracted from the bark, which also contains tannins (Thulin, 1989).

An extract of the root bark collected from Malawi in 1988 contained saponins and showed anti-inflammatory activity. In West Africa, the bark is regarded as anthelmintic, especially against tapeworms, and has long been used in a similar way in Ethiopia and Somalia, hence the specific name. Extensive tests carried out under controlled conditions have shown that the bark is, in fact, effective against tapeworm infestation. It seems to be more successful in powdered form than as a decoction, and treatment has produced no unpleasant side effects (Dery *et al.*, 1999).

It has also been reported that *A.anthelmintica* is being used as traditional medicine for gonorrhea and syphilis. Moreover, the alcoholic extract of the stem bark showed an anti gonococcal activity (Chhabra *et al.*, 1989 cited in Dawit Abebe *et al.*, 2003). In addition to the treatment of gonorrhea, in Ethiopia this plant is utilized by traditional health practitioner to treat: back pain, rheumatic pain, TB, pneumonia, chest pain, malaria, joint pain, wound in fracture, abdominal pain, muscular pain and testicular pain in Somali region Filtu. This in formation was obtained from ethno botanical survey information that had been carried out by Pasturalist Concern Association Ethiopia (PCAE) (personal communication). It was, therefore, assumed that these plant spp. and their products may also be effctive against some of the uropathogenic bacterial species and deemed necessary to screen them for this purpose against the common isolates of urinary tract infections.

4. OBJECTIVES OF THE STUDY

The General objective:

This study is thus attempted with the general objective to evaluate the *in vitro* antibacterial activity of two traditionally used medicinal plants i.e. crude and fractionated extract of *Albizia gummifera* and *Albizia anthelmintica* against four Gram-negative bacterial pathogens of urinary tract infection of adult namely *E.coli, Klebsiella spp., Pseudomonas spp.*and *Proteus spp.*

The specific objectives:

- To identify the type of bacterial uropathogens for each urine sample that was collected from out-patients
- To determine the susceptibility pattern of isolates to the commonly prescribed antibiotics
- To evaluate minimum inhibitory concentration (MIC) of crude and fractionated plant extracts of the selected medicinal plant species against *E. coli, Klebsiella spp., Pseudomonas spp.* and *Proteus spp.*
- To compare the efficacy of antibacterial activity of fractionated plant extract with the selected standard antibiotics against reference strains and clinical isolates
- To identify and recommend the promising medicinal plants for further investigation through an *in vivo* experiments

5. MATERIAL AND METHODS

5.1 Study Design

The study was carried out at Clinical Bacteriology Laboratory and Drug Research Department Laboratory of Ethiopian Health and Nutritional Research Institute (EHNRI) in collaboration with Biology Department of Addis Ababa University. After preliminary assessment of the flow of patients in the selected health institutions, two sites were selected for urine sample collection. Our sample collections were EHNRI Reception of the Core Laboratory Service and the Arsho Medical Laboratory.

5.2 Study Subject (Patients)

The study involved a total of 241 adult out-patients who were referred from St.Paul Hospital to the EHNRI Reception and those referred from different Hospitals and other Health Institutions to the Arsho Medical Laboratory over a period of five months (May 2005- September, 2006).

All patients of the study subjects were enrolled after having signed written informed consent (Appendix 1). They were then clinically confirmed for having symptoms of UTIs by an experienced physician. All the relevant data (demographic, clinical, and laboratory data) were recorded for each subject by an experienced professional according to the pre-tested Questionnaire and laboratory data sheets prepared for this purpose (Appendix .2).

For this study purpose, UTI is considered when a patient presents with one or more of the following symptoms: feeling to urinate frequently (urgency), a painful burning sensation (dysuria), supra public pain/ flank pain with fever (discomfort or pressure in the lower abdomen), cramping in the pelvic area or back and cloudy urine, or contain blood (usually, haematuria, with unpleasant smell). Patients treated with antimicrobials during the preceding one-week were excluded.

5.3 Ethical Consideration

The M.Sc research project was approved by Addis Ababa University, the Department of Biology, and Research and Ethical Clearance Committee (RECC) of EHNRI and was ethically cleared. Written informed consent was obtained from all patients participating in the study (Appendix.1).

5.4 Sample Collection and Transport

Freshly voided mid stream urine samples 10-20ml were collected from all out patient of both sample collection sites in a wide mouth sterile container after cleansing the genitals with soap and water. The urine specimens were then immediately delivered to the respective laboratory and processed with in one hour. All inoculated and cultured urine specimens at Arsho Medical Laboratory and all fresh samples collected at EHNRI Reception were brought to the Clinical Bacteriology Laboratory of EHNRI, where all the laboratory investigation were performed for Isolation, Identification and Sensitivity screening of pathogens in each specimen.

5.5 Laboratory Investigation

5.5.1 Culture and Identification

Immediately after sampling, the urine specimens were primarily inoculated on Blood agar (with 5% defibrinated sheep blood) and MacConkey agar (Oxoid, Ltd, Basingstoke, Hampshire, England) by using calibrated loop (0.001ml). Urine cultures were incubated in aerobic atmospheres at 37 ^{0}C for 24 hours.

A positive test result was defined as urine sample that contains colony count $\geq 10^5$ cfu/ml for MSU which is considered to be of significant bacteriuria (Cheesbrough, 2000; Kalhmeter, 2003; Wilson and Gaido, 2004). Positive urine cultures were further identified based on their colony morphology appearances on the media and biochemical reaction profiles, using the standard procedures (Bauer and Finegold, 1990; Cheesbrough, 2000) which routinely include; Indole, Kliger Iron agar, Lysine Iron agar, Urease, Citrate, Motility, Glucose broth, Mannitol broth, Oxidase and Catalase tests. All the isolated bacterial uropathogens were then stored at -20^{0}C in Trypto Soya broth (TSY broth) (OXOID) with 25% glycerol for further studies.

5.5.2. Antimicrobial Susceptibility Testing

The Antimicrobial susceptibility tests of bacterial uropathogens was determined using the disc diffusion method as described by WHO recommended to fulfill the National Committee for Clinical Laboratory Standards modified as Kirby Bauer disc diffusion technique (Cheesbrough, 2000). Each isolate was taken from the freezing medium and sub cultured on MacConkey Agar (OXOID) and incubated in aerobic atmospheres at 37 ^{0}C for 24 hours.

Once a pure culture was obtained, three to five similar colonies were picked and transferred with inoculation needle in to 4ml of MHB. Then the broth cultures were incubated at 37^{0}C for 2-6 hours to develop turbidity equivalent to the 0.5 McFarland turbidity standards. A sterile broth, if required, was used as diluents to obtain turbidity equivalent to the 0.5 McFarland turbidity standards.

Within 15 minutes a sterile swab was dipped in to the properly adjusted inoculum and the excess was removed by gentle rotation of the cotton swab against the surface of the tube. To obtain even inoculation, the entire MHA surface was swabbed uniformly by the cotton swab. The inoculated plates were left at room temperature for 3-5 minutes, to allow for any surface moisture to be absorbed before applying the drug disc.

Using aseptic precautions, all the selected antibiotic discs were applied on MHA by means of dispenser and then the plates were incubated at 37^{0}C for 24 hours. All isolates were tested against the following commonly used drugs: Ampicillin (10µg), Cephalothin (30µg), Chloramphenicol (30 µg), Gentamicin (120µg), Kanamycin (30µg), Naldixic acid (30µg), Nitrofurantoin (300µg), Polymyxin B (300u), Streptomycin (300µg), Tetracycline (30 µg), Co- trimoxazole (25µg) and Amoxycillin (30 µg).

Inhibition zone diameters were measured to the nearest millimeter with a slide gauge. *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. Test results were interpreted according to the most recent version of the national committee for clinical laboratory standards (NCCLS, 2002).

5.6 Plant Material

5.6.1 Collection and Identification

The plant parts of *Albizia gummifera* (seed) and *Albizia anthelmintica* (seed) used in this study were collected in August 2005. *Albizia gummifera* were collected from the southern part of the country approximately 540 km away from Addis Ababa around Bedelle and Bale regions. While *Albizia anthelmintica* were collected from the Eastern part of the country approximately 515 Km away from Addis Ababa around DireDawa Town. A taxonomist using standard Floras identified plants, and voucher specimens *A.gummifera* (AG-2110) and *A.anthelmintica* (AA-2111) were deposited in the herbarium of the Department of Drug Research, EHNRI, Addis Ababa.The plants were selected for further investigation findings based on the indigenous knowledge (Ethno medical information) and the effects observed on the crude hydro alcoholic extracts of *Albizia gummifera* against the reference strains of *E. coli* ATCC 25922 (Dawit Abebe *et al.*, 2003; Aberra Geyid *et al.*,2005).

5.6.2 Plant Extract Preparation

5.6.2.1 Crude extracts and Solvent Solvent Fractionation

The plant samples (seeds) were air-dried and ground to powder. A 300g powdered seed of *A.gummiftera* and *A.anthelminatica* were extracted by maceration and percolation with 80% methanol at room temperature. The extracts were then filtered through folded filter paper (Whatman No 1 paper) and concentrated under reduced pressure in rotary evaporator at 40^0C the gummy residue was further dried in a water bath until the methanol was removed. About 50g of methanol extracts (crude extract) were obtained from each plant samples and kept in tightly stoppered bottle in a refrigerator until it is used for antimicrobial testing and further fractionation.

Twenty-five gram of each 80% methanol extracts (crude extracts) were taken and suspended in 200ml of distilled water. The solution was transferred into a 500ml separatory funnel and shaken after addition of 50 ml chloroform three different times. The solvent layers were then allowed to separate. The chloroform layer was collected in a flask separately and filtered through filter paper in to a 250ml round bottom flask. The water suspension residue was further partitioned with n-butanol to obtain n-butanol fraction. The chloroform fraction and n-butanol fraction were reduced to dryness on a rotary evaporatory at 40^0C and

the residues were used for antimicrobial activity. Finally, the aqueous layer was filtered and lyophilized to get a dried amorphous solid, which was considered as aqueous residue lyophilized fraction (Figure 1).

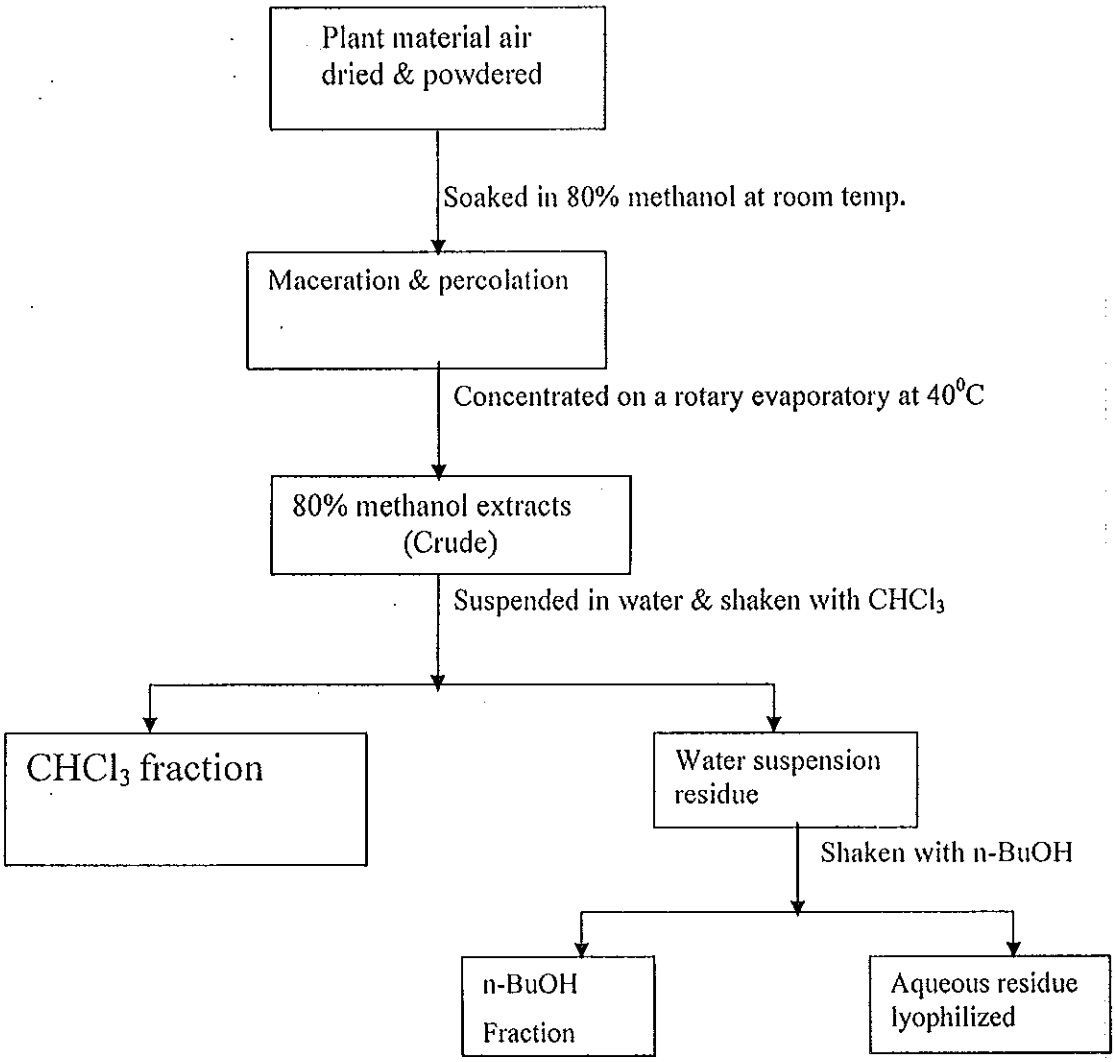


Fig.1 Flow chart for fractionation of the crude plant extract for *in vitro* antibacterial testing with some modification. (Adapted from Mahasneh and El-oqlah, 1999)

Stock solutions of standard drugs and both plant extracts were prepared using different solvents. Using analytic balance 200mg of plant crude and fractionated extracts were weighed and then both plant extracts were dissolved using 2ml of 9 % ethanol and 8 ml of distilled water in test tubes to obtain 10ml stock

solution. Similarly equal amount of standard drugs (384 mg) of Tetracycline and Amoxycillin were weighed and dissolved in different solvent to make 10ml of stock solution. In Amoxycillin stock solution preparation, saturated sodium bicarbonate was used as a solvent while in Tetracycline distilled water was utilized. In both standard drugs sterile distilled water was used as diluents. All stock solutions were stored in refrigerator at -20^0C prior to use (Andrews, 2001).

5.7. Preparation of Inocula and Culture Media

Since all clinical isolates and reference organisms were non-fastidious and facultatively anaerobic bacteria MHA (OXOID) was used without any enrichment to determine minimum inhibitory concentration (MIC) for both plant extracts and standard antibiotics. A stock solution for each plant extract and standard antibiotic was serially diluted two fold in sterile distilled water and 2 ml of each dilution was incorporated in 18 ml of MHA, which was cooled to 50^0C. The final concentration of extracts and antibiotics in the medium was determined based on the original concentration in the stock solution. Moreover, plates containing medium with 2 ml sterile distilled water were used as positive control. A negative control was also prepared using a medium without the test compounds but only with solvent.

Before inocula preparation all clinical isolates and reference organisms were subcultured on MacConkey agar. Growth method was applied as described by Andrews (2001). In short, three to five similar colonies were transferred with a sterile loop in to 4 ml MHB and then incubated at 35^0C - 37^0C until the visible turbidity was $\geq$ 0.5 McFarland standard. The suspensions were again adjusted to the 0.5 Mcfarland standard to obtain between 10^7 and 10^8 cfu/ml using sterile MHB. These suspensions were further diluted to 1:10 in MHB so as to use as starting inocula (10^7cfu/ml) for the test. Using a multipoint inoculator, 10^4 cfu/spot of suspension was delivered on to the surface of agar plate containing the plant extracts and standard antibiotics. Before incubation, the inocula were allowed to be absorbed in to the agar plate and eventually all the media were incubated at 37^0C in air for 18-20 hours (Andrews, 2001; Santo, *et al.*, 2002).

5.8 Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extracts and Standard Antibiotics

In this study, agar dilution method and conventional serial dilution were employed in the determination of MIC for both plant extracts and standard antibiotic as the method described in the Andrew's (2001). After incubation, the MIC was examined by direct visual comparison of the test cultures with the control cultures. The MIC was recorded by considering the lowest concentration of plant extracts and standard antibiotics that completely inhibited the visible growth of microorganisms. The growth of one or two colonies or fine film of growth was discarded (Andrews, 2001). All the tests were carried out in triplicate and hence the modal MIC was determined (Bosio *et al*, 2000).

5.9. Data Analysis

SPSS 10.version statistical software package was used for statistical analysis of antibiotic resistance percentage and multiple drug resistance patterns. All the results were interpreted based on the triplicates done for each plant extracts and standard antibiotics against the clinical isolates and reference strains (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853). For MIC data analysis was done taking the percentage of the modal growth inhibition value obtained for each of the 31 clinical isolates of *E. coli*, 8 of *Klebsiella spp.*, 3 of *Pseudomonas spp.*, 3 of *Proteus* spp. and both of the control strains.

6. RESULTS

6.1 Collections and Identification of Specimens

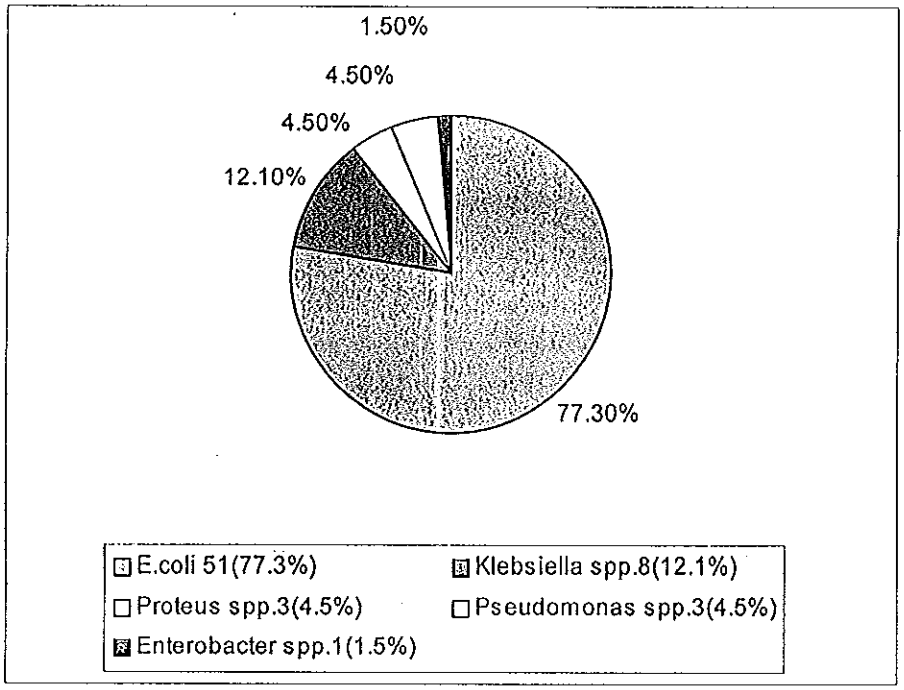
A total of 241 study subjects (age range 17-49yrs) with symptoms of UTI were investigated during the study period. Of these, 200(83%) patients were from Arsho Medical Laboratory and the remaining 41(17%) patients were from EHNRI. Distribution of study subjects by sex is shown in Table 2. Of the 241 out-patients, 158 (65.6%) were females with mean age 31.17 and 83 (34.4%)were male with a mean age 34.39. Of the total patients 6 (2.4%) gave history of urinary catheterization and all of them were culture positive.

Table2. Sex distribution of patients investigated for UTIs in Arsho Medical Laboratory & EHNRI Bacteriology Laboratory.

Study site	**Female n=158(65.6%)**		**Male n=83(34.4%)**		**Total n=241(100)**		**Total Samples**
	Positive	Negative	Positive	Negative	Positive	Negative	
Arsho Medical Laboratory	38 (30.15%)	88 (69.84%)	13 (17.56%)	61 (82.43%)	51 (25.5%)	149 (74.5%)	200 (83%)
EHNRI Bacteriology	12 (37.5%)	20 (62.5%)	3 (33.33%)	6 (66.66%)	15 (36.6%)	26 (63.4%)	41 (17%)
Total	50 (31.64%)	108 (68.35%)	16 (19.27%)	67 (80.72%)	66 (27.38%)	175 (72.61%)	241 (100%)

Occurrences of UTIs among out patients are indicated in Table 2. Significant bacteriuria was observed in 66 (27.38%) urine samples. The over all prevalence of bacterial uropathogens is higher in females 50 (31.64%) than in males 16 (19.27%). The types of bacterial uropathogens and their frequency of isolation from MSU samples are shown in Figure 2. Almost all isolates belonged to Gram-negative enteric bacteria. The most frequently isolated species was *E.coli* (77.3%), followed by *Klebsiella spp* (12.1%), and each *Proteus spp* and *Pseudomonas spp* accounted 4.5% and species of *Enterobacter* (1.5%) was recorded. In this work, no more than 2 bacterial species with two highest counts were isolated in the case of mixed cultures.

Figure 2. Types of bacterial uropathogens isolated from MSU samples collected in Arsho Medical Laboratory & EHNRI Bacteriology Laboratory Addis Ababa



6.2. Antimicrobial Susceptibility Testing

Antibiotic sensitivity and resistance profile of isolated pathogens against 12 antibiotics is presented in Appendix.3, and drug resistant strains and multiple drug resistance pattern (antibiogram) are indicated in Table 3 and 4, respectively.

Table 3. Drug resistant strains of common bacterial uropathogens

Isolates	No	Amx %	Amp %	Cep %	Chl %	Cot %	Gen %	Kan %	Nal %	Nit %	PB %	Stp %	Ttc %
E.coli	51	62.7	60.8	19.6	29.4	43.1	5.9	9.8	15.7	3.9	-	37.3	52.9
Entrobacter spp	1	100	-	-	-	-	-	-	-	100	-	-	-
Pseudomonas spp.	3	100	100	100	100	100	66.7	100	100	100	-	100	100
Proteus spp.	3	100	100.0	66.7	66.7	66.7	33.3	-	66.7	100	100	66.7	100
Klebsiella spp.	8	87.5	75.0	25.0	25.0	25.0	25.0	12.5	-	37.5	-	37.5	25.0
Total	66	69.7	64.6	22.7	33.3	43.9	12.1	13.6	19.7	18.2	4.5	40.9	53.0

Abrevations: Amx=Amoxycillin Amp=Ampicillin, Cep=Cephalothin, Chl=Chloramphenicol, Cot=Co-trimoxazole,Gen=Gentamicin,Kan=Kanamicin, Nal=Nalidixic acid, Nit=Nitrofurantoin, PB=Polymyxin B, Stp=Streptomycin, Ttc=Tetracycline

Extremely the highest resistance against most of the antibiotics (100%) were found in *Pseudomonas spp.*, except for Polymyxin B (0%) and Gentamicin (66.7%). The second high percent of drug resistance values were found in *Proteus spp*, 100% for Amoxycillin, Ampicillin, Polymyxin B, Tetracycline and Nitrofurantoin while 66.7% for Co-trimoxazole, Cephalothin, and Chloramphenicol, Streptomycin and Naldixic acid. In the case of *Klebsiella spp.* the highest rate of resistance was observed for Amoxycillin (87.5%), followed by Ampicillin (75%), and Nitrofurantoin (37.5%). For *E.coli* the four highest resistance rates were seen in Amoxycillin (62.7%), Ampicillin (60.8%), Tetracycline (52.9%) and Co- trimoxazole (43.1%). *Enterobacter spp.* showed highest sensitivity at 100% for all antibiotics except for Nitrofurantoin and Amoxycillin at (100%) resistance to each of them. Out of the 66 uropathogen total bacterial isolates tested against the 12 antibiotics, 41(62.1) revealed multiple resistance to at least two combined drugs while 3(4.5%) of them revealed the highest combination of multiple drug resistance, to atotal of 11 out of 12 drugs tested (Table4).

Over all highest sensitivity percent was observed in all isolates for Polymyxin B(≥ 96.1%) except in *Proteus spp.* (0%). The next four highest susceptibility rates was also observed in the case of *E.coli* for Gentamicin (94.1%), Nitrofurantoin (88.3%), Kanamicin (86.3%) and Naldixic acid (84.3%). Though the number of isolates was low, multiple drug resistances were high in *Pseudomonas spp.* and *Proteus spp.* (Table 4). Between 3(4.5%) and 41(62.1%) isolated pathogens showed resistance from 2 to 11 antimicrobials and 3(4.5%) isolates were resistance to only one antibiotic. Only 7(10.6%) showed no resistance to the 12 antibiotics. However, no isolates that showed resistance to all 12 antibiotics were also recorded (Table 4).

Table 4. More frequent Antibiogram pattern in bacterial uropathogens

Antibiogram pattern	Types of Bacterial uropathogens					
	E.coli 51(100%)	*Entrobacter* 1(100%)	*Klebsiella* 8(100%)	*Proteus* 3(100%)	*Pseudomonas* 3(100%)	Total 66(100%)
R0: Sensitive to all antibiotic	7(13.7%)	0	0	0	0	7(10.6%)
R1: Resistant to only one antibiotics	3(5.9%)	0	0	0	0	3(4.5%)
R2: AmxAmp	29(56.9%)	0	6(75%)	3(100%)	3(100%)	41(62.1%
R3: AmxAmpTtc	22((43.1%)	0	2(75%)	3(100%)	3(100%)	30(45.4%
R4: AmxAmpTtcCot	17(33.3%)	0	0	3(100%)	3(100%)	23(34.8%
R5: AmxAmpTtcCotStp	16(31.4)	0	0	2(66.7%)	3(100%)	21(31.8%
R6: AmxAmpTtcCotStp Chl	9(17.6%)	0	0	2(66.7%)	3(100%)	14(21.2%
R7: AmxAmpTtcCotStp ChlCep	5(9.8%)	0	0	2(66.7%)	3(100%)	10(15.1%
R8: AmxAmpTtcCotStp ChlCepNal	2(3.9%)	0	0	2(66.7%)	3(100%)	7(10.6%)
R9: AmxAmpTtcCotStp ChlCepNal Nit	1(2%)	0	0	2(66.7%)	3(100%)	6(9%)
R10: AmxAmpTtcCot StpChlCepNalNitKan	1(2%)	0	0	0	3(100%)	4(6%)
R11: AmxAmpTtcCot StpChlCepNalNitKanGen	1(2%)	0	0	0	2(66.7)	3(4.5%)

Abbrevations: R2-R11: resistant to two to eleven antibiotics.

* See table .3 for antibiotics abbreviations

6.3. Determination of Minimum Inhibitory Concentration (MIC) for Plant extracts and Standard Antibiotics

A total of 45 bacterial pathogen among our 66 clinical isolates, and two reference strains, *E.coli* and *P. aeruginosa* ,were included in this study to see the *in vitro* antimicrobial activities of plant extracts and susceptibility of standard antibiotics. Out of the 45 clinical isolates 31 were *E.coli*, which were selected based on their multiple drug resistance that revealed a resistance pattern for ≥ two multiple antibiotics. The remaining clinical isolates were *Klebsiella* (8 *spp.*), *Pseudomonas* (3 *spp.*) and *Proteus* (3 *spp.*).

6.3.1. MIC for The Crude 80% methanol extracts of *Albizia gummifera* and *Albizia anthelmintica*

The crude 80% methanol extracts of the seeds of *Albizia gummifera* and *A. anthelmintica* were solubilized by 9% ethanol and sterile distilled water and then were tested against the selected clinical isolates and reference strains. Both crude 80% methanol extracts of the plants indicated antibacterial activities against all the selected clinical isolates of *E. coli* and *Klebsiella spp.* whereas no antibacterial activities were observed in *Proteus spp.* and *Pseudomonas spp.*, even at 2000 µg /ml minimum concentration. The detail results of the *in vitro* susceptibility of the test organisms to the crude extracts were indicated in appendix 4 -9.Crude extracts of both *A. gummifera* and *A. anthelmintica* exhibited equal MIC (500µg/ml) result against the clinical isolates and reference strain of *E.coli* (Table 5).

Table5. MIC for 80% crude methanol extracts against clinical isolates and reference strains

Clinical Isolates & Standard organisms	Crude extract of *A. gummifera* MIC (µg/ml)	Crude extract of *A.anthelmintica* MIC (µg/ml)
E. coli 31(100%)	500	500
E. coli ATCC 25922	500	500
*Klebsiella spp.*8 (100%)	2000	2000
*Pseudomonas spp.*3 (100%)	NI	NI
P.aeruginosa ATCC 27853	NI	NI
*Proteus spp.*3 (100%)	NI	NI

NB NI: No inhibition at 2000µg/ml

6.3.2. MIC for The Semi Purified Fractions of *A.gummifera* and *A.anthelmintica* Against Clinical Isolates and Reference Strains

Over all semi purified fractions of plant extracts revealed a better antimicrobial activity against the clinical isolates and reference strains as low as 250 µg/ml and 125 µg/ml respectively (Tables 6 and 7 and Figures 3 and 4). However, chloroform fraction didn't exhibit any antibacterial activity against all clinical isolates and reference strains. Aqueous solubilized fraction of both plants showed equal antibacterial activity; the MIC was 1000 µg/ml and 500 µg/ml for clinical isolates of *E. coli* and *E. coli* ATCC 25922, respectively (Figures 3 and 4). In case of *Klebsiella spp.* 87.5% of them exhibited the MIC at 2000 µg/ml of aqueous solubilized fraction of *A. gummifera* while 100% for *A. anthelmintica* (Tables 6 and 7 and Figure 5). Likewise, the crude extracts, the aqueous solubilized fraction didn't indicate any antibacterial activity at 2000µg/ml for *Pseudomonas spp.*, including reference strains and *Proteus spp.*(Tables 6 and 7).

Table 6. MIC for semi purified fraction of *Albizia gummifera* against clinical isolates and reference strains.

Clinical Isolates & Standard organisms	MIC (µg/ml) for aqueous solubilized fraction	MIC (µg/ml) for n-butanol fraction
E. coli 31(100%)	1000	250
E. coli ATCC 25922	500	125
*Klebsiella spp.*8 (100%)	2000*	1000
*Pseudomonas spp.*3 (100%)	NI	NI
P.aeruginosa ATCC 27853	NI	2000
*Proteus spp.*3 (100%)	NI	NI

NB *: Only for 87.5% of clinical isolates of *Klebsiella spp.*
NI: No inhibition at 2000µg/ml

Table 7. MIC for semi purified fraction of *Albizia anthelmintica* against clinical isolates and reference strains

Clinical Isolates & Standard organisms	MIC (µg/ml) for aqueous solubilized fraction	MIC (µg/ml) for n-butanol fraction
E. coli 31(100%)	1000	250
E. coli ATCC 25922	500	125
Klebsiella spp. 8 (100%)	2000	500
Pseudomonas spp. 3 (100%)	NI	2000*
P.aeruginosa ATCC 27853	NI	2000
Proteus spp. 3 (100%)	NI	NI

NB * Only for 66.7% of clinical isolates of *Pseudomonas spp.*
NI: No inhibition at 2000µg/ml

Generally, in this investigation the reference strains were relatively more sensitive than the clinical isolates and the least MIC value was recorded (125 µg/ml). Moreover, It had been observed that n-butanol fraction of plant extracts exhibited antibacterial activity against particularly reference strains *P. aeruginosa* ATCC 27853 and the clinical isolates of *Pseudomonas spp.* (66.7%) with the MIC value of 2000 µg/ml for *A.anthelmintica* (Tables 6 and 7).

6.3.3. MIC for Standard Antibiotic Against Clinical Isolates and Reference Strains

In this study, in order to have a better view on the antibacterial activity of plant extracts, a comparison was made with standard antibiotics namely Tetracycline and Amoxycillin as a positive control. These antibiotics were selected based on the result obtained from Disc diffusion, antimicrobial susceptibility testing i.e those antibiotics which were highly resisted by clinical isolates and drugs that; are relatively cheap cost, commonly prescribed and easily available in community. The detailed results are indicated in Appendix 10 and 11. The MIC value was range from 2.5 - 320 µg/ml and 7.5 - 3840 µg/ml for Tetracycline and Amoxycillin respectively (see Table 8).

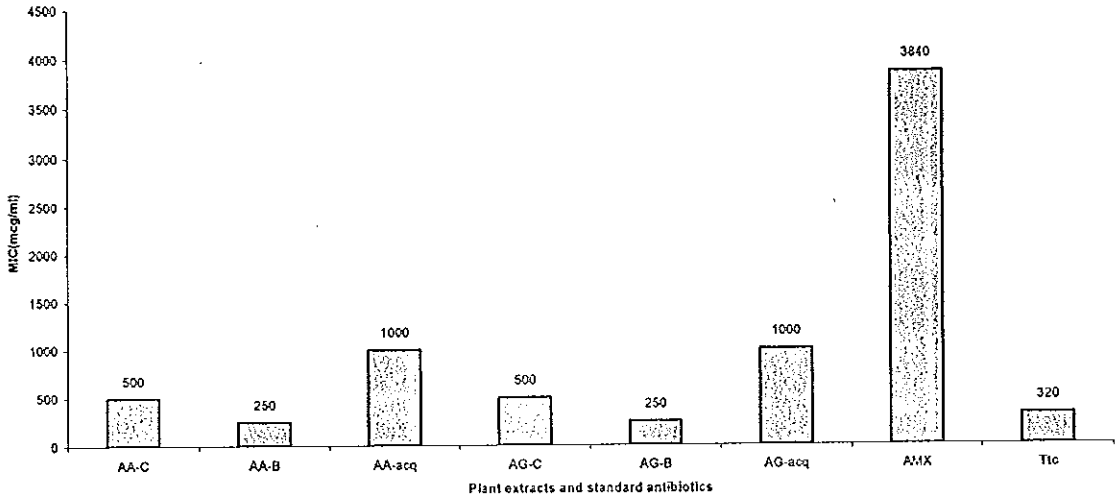
Table 8. MIC values of n-butanol fraction of *A. gummifera* & *A. anthelmintica* compared to

Clinical Isolates & reference strains	***A.gummifera*** (µg/ml)	***A.anthelmintica*** (µg/ml)	Tetracycline (µg/ml)	Amoxycillin (µg/ml)
E. coli 31(100%)	250	250	320	3840
E. coli ATCC 25922	125	125	2.5	7.5
Klebsiella spp. 8 (100%)	1000	500	320	3840*
Pseudomonas spp. 3 (100%)	3840	2000**	320	3840
P.aeruginosa ATCC 27853	2000	2000	40	960
Proteus spp. 3 (100%)	NI	NI	320	3840***

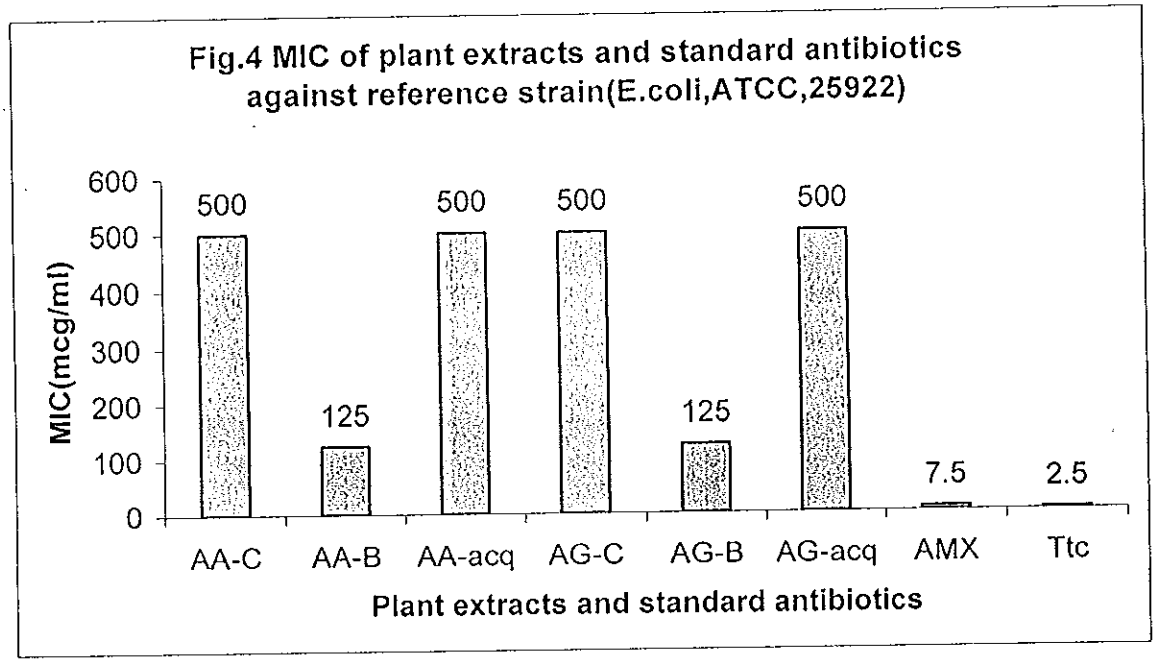
the Standard antibiotics against tested organisms.

NB *: only for 87.5% of clinical isolates of *Klebsiella spp.*
**: only for 66.7% of clinical isolates of Pseudomonas spp
***: only for 66.7% of clinical isolates of *Proteus spp.*
NI: No inhibition at 3840(µg/ml)

Fig.3 MIC of plant extracts and standard antibiotics against clinical isolates of E.coli

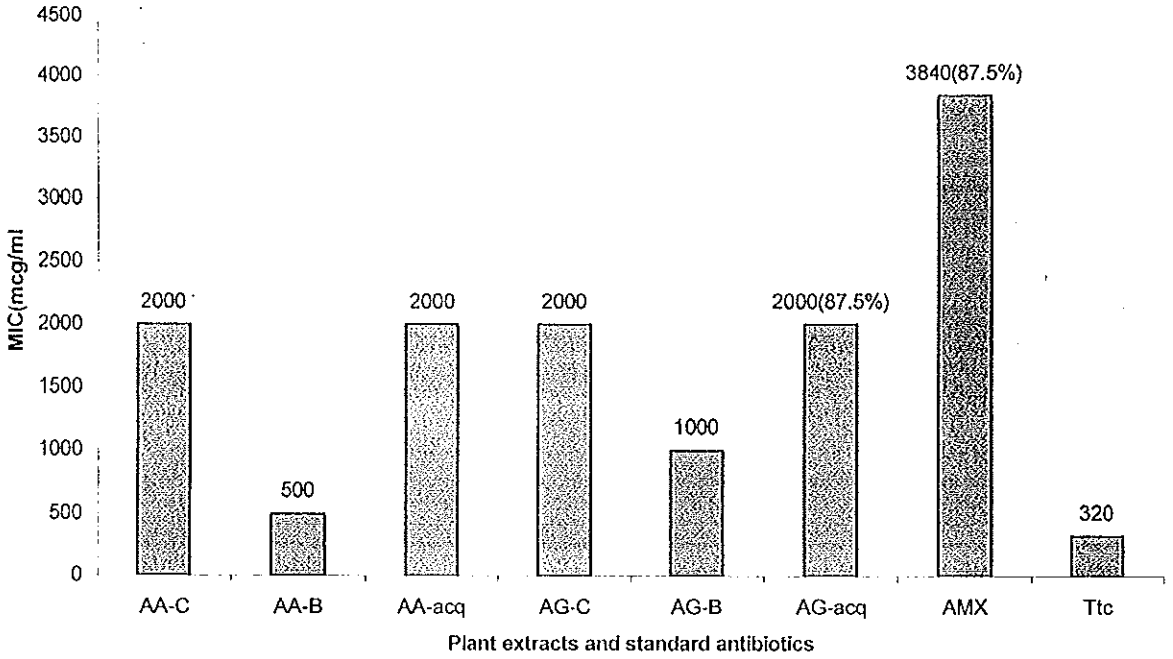


NB: AA-C= *A.anthelmintica* crude extract, AA-B=*A.anthelmintica* n-butanol fraction,
AA-acq= *A.anthelmintica* aqueous solubilized fraction,AG-C= *A.gummifera* crude extract
AG-B= *A.gummmfera* n-butanol fraction,AG-acq= *A.gummifera* aqueous solubilized fraction
AMX=Amoxycillin,Ttc=Tetracycline.



NB: See Fig.3 for plant extracts & antibiotics abbreviations

Fig.5 MIC of plant extracts and standard antibiotics against clinical isolates of Klebsiella spp.



NB: See Fig.3 for plant extracts & antibiotics abbreviations

7. DISCUSSION

In this study, the results showed that the etiological pathogens of UTIs almost all belong to Gram-negative enteric microorganisms (Fig.2). A retrospective study of urinary pathogens isolate from Tikur Anbasa Hospital (Dawit Wolday and Worku Erge, 1997) reveals that these Gram-negative bacteria comprise approximately 95% of all isolates. The same work also reported that *E. coli* and *Klebsiella spp.* were the most common organisms. Even in the recent literature Wilson and Gaido, (2004) indicated that aerobic and facultative Gram-negative bacteria cause nearly all UTIs in out patients.

Our studies also indicated that *E.coli* (77.3%) is still the most common cause of community acquired UTIs. This corresponds with the data obtained by other investigators in Ethiopia (Messele Gedebou, 1983; Dawit Wolday and Worku Erge, 1997) and elsewhere in the world (Kadri *et al.*, 2002;Gupta, 2003; Kalhmeter, 2003). *Klebsiella* was the second most frequently isolated urinary pathogens as reported previously (Mesele Gedebou, 1983). Our result also showed a similar finding.

Clinical isolates of *Proteus* and *Pseudomonas* were also found in this study from patients who gave history of previous catheterization. According to Dawit Wolday and Worku Erge (1997) study, these bacteria were also isolated most frequently from patients in surgical ward. Moreover, this idea is supported by other literatures (Cheesbrough, 2001; Stamm, 2001) indicating that urinary tract infections caused by *Pseudomonas* and *Proteus* are associated with hospital acquired infections, often following catheterization or gynecological surgery.

In the present study, the prevalence of UTI is higher in females (31.6%) than in males (19.3%). Similar results have been reported in Ethiopia (Messele Gedebou, 1983; Dawit Wolday and Worku Erge, 1997) and elsewhere in the world (Madigan and Neff, 2003). The higher incidence of UTI in women is suggested to be due to the shortness of the female urethra, which is one and one half inches compared to eight inches in men. Bacteria from fecal matter can also be easily transferred to the vagina or the urethra (Salyers and Whitt, 1994; Stamm, 2001).

High rate of infection in sexually active women is suggested since sexual intercourse causes the introduction of bacteria in to the bladder and is temporarily associated with the on set of cystitis (Stamm, 2001). Moreover, use of spermicidal coated condoms, inhibits growth of a major components of the resident microflora and thus helps to create favorable environment for colonization of the vagina by uropathogenic *E.coli* strains (Salyers and whitt, 1994).

In our study UTI, in the vast majority of patients, is caused by a single species, but occasionally there may be a mixed infection. Some microbiologists regarded urine culture with polymicrobial (mixed) growth as contaminants (Denman and Greenough, 1991). However, polymicrobial growth from midstream urine has been found among patients with confirmed bladder infection. Mixed infections are more likely to occur with underlying disorders that interfere with free urine flow and are frequent also among patients with indwelling catheter (Denman and Greenough, 1991). Thus, in the present study mixed culture was not isolated since all urine samples were obtained exclusively from non-catheterized patients except those who gave history of catheterization.

Although the sample size in the present study is relatively small, the information obtained from the study cannot be over looked since it revealed the extent of antimicorbial resistant bacterial infection in community acquired UTI. In this study most of the bacterial isolates between 4.5% and 62.1% of all isolates and between 2.0% and 56.9% of *E.coli* were multiple drug resistant strains (i.e. resistant to two or more antimicrobial agents) (Table 4). The other researcher (Moges *et al.* 2002) also reported that above 68% of isolated pathogens were multiple drug resistant.

Given that *E.coli* is the principal pathogen in urinary tract infections, particularly among out patients, resistance to Amoxycillin, Ampicillin, Tetracycline, Co-trimoxazole,and Streptomycin is an important indicator of whether these antibiotics should continue to be used empirically or not. The present study, of 51 *E.coli* isolates from out patients confirms that resistance to Co-trimoxazole, Tetralycline, Ampicillin, and Amoxycillin is 43.1, 52.9, 60.8, and 62.7%, respectively (Table 3). According to Moges *et al.*, (2002) studies also indicated that the highest resistance rates were observed in Ampicillin (69.21%), Tetracycline (68.0%) and Co-trimoxazole (56.4%) for 78 *E.coli* isolates. Similar results also observed in the study conducted in Jimma Hospital (Zeleke Wolde Tensaye, 2002) and Yirgalem Hospital Southern Ethiopia (Ashenafi Beilu and Lindtiorn, 1999).

The possible explanation for such kinds of resistance is that most of these drugs are easily available and may be used in discriminately. Recent studies conducted by Kalhmeter *et al* (2003) indicated that there were strong and statically significant relationships between total antimicrobial consumption and the incidence of multiple drug resistant strains of *E.coli*. From a microbiological point of view and based on the results of this study and others it would seem reasonable to reconsider not to use of Co-trimoxazole, Tetracycline, Amipicillin and Amoxycillin for the treatment of UTI.

Over all highest resistance pattern was observed for species of *Pseudomonas* and *Proteus* isolated from patients who gave history of previous catheterization. For these patients, it could be suggested that they might have acquired UTI from hospital during catheterization. This is also supported by a study performed in Gonder (Moges *et al*, 2002).

Nitrofurantoin, Naldixic acid, Kanamicin, Gentamicin and Polymyxin B are the drug that showed effectiveness against all isolates for 74.2% - 92.4% (susceptibility value) (Appendix 3). This also corresponds to the result reported by other investigators (Dawit wolday and Worku Erge, 1997 and Moges *et al.* 2002). Low frequency of resistance in these antibiotics is observed, perhaps, because that the agents might have not been commonly used due to their high cost and not easily available in the community. However, the extensive use of these antibiotics in the future appears to lead to problems with the development of resistance and eventually limits its efficacy. This, therefore, suggests the need to look in to various alternatives challenging these multidrug resistant bacterial UTI agents. Among the possible alternatives is to search into the effects of some traditionally used plant extracts against these bacterial agents. Thus, that is why the objectives of such studies should be the selection of plants like *A. gummifera* and *A. anthelmintica* for the purpose of screening their effects against the isolated uropathogenic strains.

As it is indicated in Table 5 and Figures 3 and 4 crude 80% methanol extracts of both *A. gummifera* and *A. anthelmintica* exhibited antibacterial activity with MIC 500 µg/ml, on both clinical isolates and reference strains of *E. coli*. The same antibacterial activity result was observed in the case of crude extract of seeds of *A. gummifera* against the standard organisms (*E coli* ATCC 25922) during the studies of screening of some medicinal plants of Ethiopia (Aberra Geyid *et al*, 2005). Similar result was also

reported for crude extract of *A.gummifera* against clinical isolates of *Streptococcus pyogenes* and *Streptococcus pneumonia* (Abayneh Unasho, 2005). Even though, the MIC value was higher (2000 µg/ml) than the previous one; in the present study antibactieral activity of crude extracts of both plants against clinical isolates of *Klebsiella spp* was also recorded (Table 5 and Figure 5). Clinical isolates of *Pseudomonas spp., P.aeruginosa* ATCC 27853 and clinical isolates of *Proteus spp.* were all resistant to all test crude extract of plants at 2000 µg/ml, (the highest concentration level tested).

As it is indicated in Table 6 and Figure 3,4, and 5, 9% ethanol solubilized semipurified fraction (n-butanol fraction) of *A.gummifera* showed antibactierial activity against clinical isolates of *E.coli* and *klebsiella spp.* including the two standard reference strains. The best activity was seen for n-butanol fraction against isolates of *E.coli* (250 µg/ml) and *E.coli* ATCC 25922 (125 µg/ml). This result also better than Abayneh Unasho's (2005) finding with MIC 500µg/ml and 1000µg/ml against reference strains and clinical isolates of *S.pneumonia,* respectively. In our investigation again good activity was also observed against isolates of *Klebsiella spp.* at MIC 1000 µg /ml and a weak activity against *P. aeruginosa* ATCC 27853 with MIC of 2000 µg/ml. However it was inactive against clinical isolates of *P. aeruginosa* and *Proteus spp.* at concentration 2000 µg/ml.

In the case of *A.anthelmintica,* best activity was also observed for n-butanol fraction against isolates of *E.coli* with MIC 250 µg/ml, and *E.coli* ATCC 25922 with MIC 125 µg/ml (Table 7 and Figures 3 and 4). Though the concentration was high, 66.7% of clinical isolates of *P.aeruginosa* and *P. aeruginosa* ATCC 27853 were inhibited at concentration 2000 µg/ml. Still *Proteus spp* was resistant to n-butanol fraction at 2000 µg/ml. This variation of the results may reflect the selectivity of the bioactive compounds of the plant extracts against different species of clinical isolates (Martin *et al*, 2004).

During the preliminary study and also in our investigation chloroform fractions containing intermediate polar compounds such as phenols, diterpenes, etc did not exhibit antibacterial activity therefore we excluded these fractions. Aqueous solubilized fraction of both plants exhibited antibacterial activities against clinical isolates of *E.coli, Klebsiella spp* and standard organism *E coli* ATCC 25922 (Table 6 and 7). This shows that there was a presence of some active compounds in aqueous phase of tested plants. Aqueous residue of lyophilized fraction contained very polar compounds like polar saponins, phenolic

glycosides and terpenes, therefore, it can be suggested that these compounds may contribute for antibacterial activity. Occasionally tannis and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Cowan, 1999).

Over all in this study n-butanol fraction displayed more antibacterial activity against the tested organisms compared with the crude and aqueous phase extracts. N-butanol fraction contained polar compounds including terpenes, saponins, phenols, etc. High effectiveness of n-butanol fraction may reflect the presence of more active ingredients in the extracts. Moreover, *A. anthelmintica* exhibited relatively better activity against the tested organisms at a lower MIC particularly for n-butanol fraction than the *A. gummifera.* This difference appear to be due to a number of factors like time of collection of plant materials, edaphic factors and climate which might in turn affect the amount of active ingredients in the plant material, and also chemical constituent of plants (Mintesnot Ashebir and Mogessie Ashenafi, 1999).

When individual organisms are compared, *E.coli* became more susceptible to this plant extracts and then followed by *Klebsiella spp..* Even though, the methods employed were varied the result obtained from antibacterial activity against *Klebsiella spp* with MIC 500 µg/ml in the present study was comparable to the findings revealed by Hirut Lemma *et al* (2002) and even better than Dawit Dikasso *et al.*, (2002). In the of Dawit Dikasso *et al.* (2002) *Allium sativum* and Hirut Lemma *et al.*(2002) *Plumbago zylanica* were investigated for antimicrobial activity and they obtained the MIC value with 24.4 mg/ml and 0.4 mg/ml against the clinical isolates of *Klebsiella pneumonia,* respectively.

N-butanol fraction of both plants showed lower MIC (125 µg/ml) against the standard test organisms *E.coli* ATCC 25922 than the clinical isolates of *E.coli.* Like wise, *Pseudomonas aeruginosa* ATCC 27853 was inhibited at concentration 2000µg/ml whereas all clinical isolates of *P.aeruginosa* were not inhibited by the same concentration (Table 6 and 7). The possible explanation for the result differences between the standard organism and clinical isolates is that the clinical isolates are basically obtained from clinically symptomatic patients. Therefore, clinical isolates may have chance of exposure to antibacterial agents that may bring change to the molecular and other factors. Hence, they are expected to be less sensitive as compared to standard organisms with no chance of exposure to the above agents.

Changes were observed in susceptibility results, at least in part, from changes in the outer cell layers that increase barrier properties and prevent access of biocides to their site of action (Fluit *et al.*, 2001;Gilbert and McBain, 2003). Thus, in searching new bioactive products it is suitable to employ plant extracts that have potency selectively active against problematic microorganisms like resistant pathogens that are isolated from clinical sites (Vanden Berghe and Vlietinck, 1991).

In order to have a better view on the antibacterial activity of n- butano fraction of both plants, comparison was made with standard antibiotics namely Tetracycline and Amoxycillin as a positive control. In the case of Tetracycline MIC value was ranged from 2.5 - 320 µg/ml, and for Amoxycillin was 7.5 -3840µg/ml (Table 8 and Appendix 10 and 11). In this investigation, clinical isolates of *E.coli* were inhibited at 320µg/ml and 3840µg/ml of Tetracycline and Amoxycillin, respectively whereas the fractionated plant extracts showed much better antibacterial activity against these clinical isolates at a lower MIC 250µg/ml compared to these antibiotics (Table 8 and Figure 3). Moreover, there were isolates, which were inhibited at 125µg/ml for 45.2 % and 61.3 % by *A. gummifera* and *A. anthelmintica,* respectively (appendix 8 and 9). This was also the best result recorded in our study against the clinical isolates.

For the remaining clinical isolates, the effectiveness of fractionated plant extracts was much lower than the Tetracycline, which inhibited all the clinical isolates at MIC 320µg/ml. Still the fractionated plant extracts excluding *Proteus spp.* exhibited much better antibacterial activity against *Klebsiella spp.* and *Pseudomonas spp.* than that of Amoxycillin which inhibited the clinical isolates at highest concentration 3840µg/ml (Table 8 and Figure 5). Such a high concentration against the clinical isolates exhibited by Amoxicillin might be related with the tested pathogens, which were Gram-negative and less sensitive to this antibiotic.

However, both standard antibiotics displayed much lower MIC value against the reference strains than the plant extracts (Table8 and Figure 4). It is difficult to conclude from this result in comparison of the antimicrobial potency of the plant extracts and standard antibiotics since a higher sensitivity may be caused by a high active compounds present in quite small amounts or by a substance of comparatively low activity but present in high concentration of plant extract (Venden Verghe and Vlietinck, 1991).

These two plants belong to the Fabaceae family and the *Albizia* genus. This may mean that the same compounds could be possibly responsible for the antibacterial activity in these plants. It has been reported from the work of Asfaw Debella *et al.* (2001) *A.gummifera* contained triterpens saponin and similarly the presence of several of these compounds in *A.anthelmintica* was studied (Carpani *et al.*, 1989 cited in Runyoro *et al.*, 2006). In this study also in order to see their similarity Thin layer Chromatography (TLC) was developed and then the presence of saponins in these plants were supported by chromatogram obtained by visualizing with vanillin–sulfuric acid (Appendix 12) in which the probable active compounds gave purple or blue colour with these reagent, a normal positive test for triterpenoidal and steroidal saponins respectively (Runyoro *et al.*, 2006). Additionally, the extracts of these plants formed strong foam particularly *A. anthelmintica*, with water during our investigation indicating that they contain saponins.

The results obtained from this study might be considered sufficient for further studies aimed at isolating and identifying the bioactive compound and evaluating possible synergism of antimicrobial activity belong to these plant extracts and then further investigation using *in vivo* experiment may address potential therapeutic agents. Moreover, recently Runyoro *et al.* (2006) reported that the root barks of *A. anthelmintica* have showed strong activity against standard strains of *Candida albicans*. It is known that *Candida albicans* is a common fungal causative agent for UTI. Therefore, this is also an indication that the plant may have a promising potential potency for both major bacterial and fungal causative agents of UTI like *E.coli* and *Candida albicans*, respectively.

8. CONCLUSIONS AND RECOMMENDATION

This investigation has indicated that *E.coli* was the most common cause of community acquired UTI in the study sites from the out patients. UTI is more common in females than males. This study also clearly indicated that high incidence of resistant to the commonly prescribed antibiotics. Most of the clinical isolates were multiple drug resistance particularly those isolated from patients gave history of catheterization. In this study as an alternative solution to combat resistant pathogens two traditionally used medicinal plants were tested against the clinical isolates and reference strains. Thus, from this investigation the following conclusive points could be extracted:

1. Both *A. gummifera* and *A. anthelmintica* contain antibacterial agents and need further purification for better efficacy;
2. n-butanol fractionated plant extracts revealed relatively more potent effect against the pathogen than other extraction solvents;
3. Relatively n-butanol fractionated plant extracts exhibited more activity against the clinical isolates of *E.coli* than both standard antibiotics;
4. Clinical and reference strains of *E.coli* were more susceptible to the plant extracts than other clinical isolates and reference strain; and,
5. It can be considered as another indication of the traditional wisdom of Ethiopian traditional health practitioners.

Based on our findings and from the above conclusions, we would like to propose the following recommendations.

1. It would be recommendable to carry out antimicrobial susceptibility patterns of bacterial uropathogens before treating the patients.
2. Despite the fact that bacterial uropathogens were multiple drug resistant to Amoxycillin, Ampicillin, Tetracycline and Co-trimoxazole in the present study, the same antibiotics are still commonly used in different Health Institutions. Therefore, it would be reasonable to reconsider the use of other alternative antibiotics for the treatment of UTIs.
3. The advantage microbes gain from their innate adaptability is augmented by the wide spread and some times in appropriate use of antibiotics. Therefore creating awareness (education) about prudent use of existing antimicrobials could slow down the development of antimicrobial resistant bacterial strains.

4. The continued evolution of antimicrobial resistance among community-acquired isolates is worrisome therefore it should be mandatory to perform further surveillance studies.
5. It would be wise to employ both clinical isolates and reference strains in the evaluation of the effectiveness of plant extracts because plant extracts that have been effective against the reference strains would not be necessarily effective against clinical isolates.

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10. APPENDICES

Appendix .1 Consent Form

(To be translated to patient's language)

Date: -------

Ward/Center: -------

I----------hereby give my consent for UTI examination and to give mid-stream urine specimens, so that Semere Kassaye can utilize the specimens for the scientific investigation titled " *In vitro* Investigation of Antimicrobial activities of *Albizia gummifera* and *Albizia anthelmintica* on Major Bacterial Uropathogens isolated from Adult patients in Addis Ababa" at EHNRI.

Appendix .2.Questionnaire,Laboratory data & Antimicrobial susceptibility test

I. Questionnaire

The objective of this questionnaire is only to assess clinical background of UTI patient.

Only volunteer's patient will be requested.

Name of the Hospital_____

Date_____

Sample code No_____

Time of specimen collection_____

1. Patient's Identification
   1.1. 1.1 Name: _____
   1.2. 1.2. Age: _____
   1.3. 1.3. Sex: male _Female _____ : Pregnant ----------
   Non-pregnant---------
2. Occupation: _____
3. Marital status: -Single------Married ----- -Divorced-----
4. Education Background:
   Illiterate _____
   Literate: -Elementary------
   -High school------
   - Graduate level-----
5. Status of a patient
   5.1. Inpatient: -Catheterized---------
   -Non-catheterized ----
   5.2. Out patient: - Previously catheterized --------
   Non-catheterized ----------
   5.3. Recurrent UTI: Yes --- No-----
   5.4. Complicated UTI: Yes--- No-----
   5.5. Exposure to antibiotics: Yes---- No---- If yes: Name of antibiotics _____
   5.6. Use of traditional medicine: yes ----- No------
   Type of herbal remedy used if any: ----------

II. Laboratory data

1.Date of urine collection: ----------

2.Type of specimen: a. Mid stream urine sample ---------
b. Catheter urine sample --------

3.Culture and Identification.

a. Significant bacteriuria : Yes:------
No: ------

b. Name of pathogen isolated ----------

III. Antimicrobial susceptibility testing

	S	I	R
1. Amoxycillin	--- ------	----------	----------
2. Ampicillin	---------	----------	---------
3. Co-trimoxazole	------	---------	--------
4. Cephalothin	-- ------	--------	--------
5. Chloramphenicol	-- -----	-------	---------
6. Gentamicin	--------	--------	---------
7. Kanamicin	--------	--------	---------
8. Streptomycin	---------	--------	---------
9. Tetracycline	---------	--------	--------
10. Nalidixic acid	---------	-------	--------
11. Nitrofurantoin	---------	--------	---------
12. Polymyxin B	---------	--------	-------

Appendix .3. Antibiotic sensitivity and resistance profile of isolated pathogens against 12antibiotics

Isolates	no	pattern	Amp	Cot	PB	Cep	Chl	Gen	Kan	Stp	Ttc	Nal	Nit	Amx
E.coli	51	S	19(37.3%)	29(56.9%)	49(96.1%)	28(54.9%)	34(66.7%)	48(94.1%)	44(86.3%)	23(45.1%)	20(39.2%)	43(84.3%)	45(88.3%)	19(37.3)
		I	1(2.0%)	-	2(3.9)	13(25.5%)	2(3.9%)	-	2(3.9%)	9(17.6%)	4(7.8%)	-	4(7.8%)	-
		R	31(60.8%)	22(43.1%)	-	10(19.6%)	15(29.4%)	3(5.9%)	5(9.8%)	19(37.3%)	27(52.9%)	8(15.7%)	2(3.9%)	32(62.7%)
Enterobacterspp.	1	S	1(100.0%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	1(100%)	-	-
		I	-	-	_	-	-	-	-	-	-	-	-	-
		R	-	-	-	-	-	-	-	-	-	-	1(100%)	1(100%)
Pseudomonas spp.	3	S	-	-	3(100%)	-	-	1(33.3%)	-	-	-	-	-	-
		I	-	-	-	-	-	-	-	-	-	-	-	-
		R	3(100%)	3(100%)	-	3(100%)	3(100%)	2(66.7%)	3(100%)	3(100%)	3(100%)	3(100%)	3(100%)	3(100%)
Proteus spp	3	S		1(33.3%)	-	-	1(33.3%)	2(66.7%)	3(100%)	1(33.3%)	-	1(33.3%)	-	-
		I	-	-	-	1(33.3%)	-	-	-	-	-	-	-	-
		R	3(100.0%)	2(66.7%)	3(100%)	2(66.7%)	2(66.7%)	1(33.3%)	-	2(66.7)	3(100%)	2(66.7%)	3(100%)	3(100%)
Klebsiella spp	8	S	-	6(75.0%)	8(100%)	5(62.5%)	5(62.5%)	6(75.0%)	7(87.5%)	4(50.0%)	3(37.5%)	6(75.0%)	4(50.0%)	1(12.5%)
		I	2(25.0%)	-	-	1(12.5%)	1(12.5%)	-	-	1(12.5%)	3(37.5%)	2(25.0%)	1(12.5%)	-
		R	6(_75.0%)	2(25.0%)	-	2(25.0%)	2(25.0%)	2(25.0%)	1(12.5%)	3(37.5%)	2(25.0%)	-	3(37.5%)	7(87.5%)
Total	66	S	20(30.8%)	37(56.1%)	61(92.4%)	34(51.5%)	41(62.1%)	58(87.9%)	55(83.3%)	29(43.9%)	24(36.4%)	51(77.3%)	49(74.2%)	20(30.3%)
		I	3(4.60%)	-	2(3.0%)	15(22.7%)	3(4.5%)	-	2(3.0%)	10(14.2%)	7(10.6%)	2(3.0%)	5(7.6%)	-
		R	42(64.6%)	29(43.9%)	3(4.5%)	17(22.7%)	22(33.3%)	8(12.1%)	9(13.6%)	27(40.9%)	35(53.0%)	13(19.7%)	12(18.2%)	46(69.7%)

Abbreviations: S=sensitive, I=intermediate, R=resistance, Amx=Amoxycillin, Amp=Ampicillin, Cep=Cephalothin, Chl=Chloramphenicol, Cot=Co-trimoxazole, Gen=Gentamicin, Kan=Kanamicin Nal=Nalidixic acid, Nit=Nitrofurantoin, PB=Polymyxin B, Stp=Streptomycin, Ttc=Tetracycline

Appendix. 4. *In vitro* antibacterial activities of crude extract of *Albizia gummifera* against clinical isolates & standard organisms

Clinical Isolates & Standard organisms	**Conc. of extracts (µg/ml)**								**Media & Solvent Control**	
	2000		1000		500		250			
	G%	I%	G%	I%	G%	I%	G%	I%	G%	I%
E.coli (31)	0	100	0	100	0	100	100	0	100	0
E.coli ATCC 25922	0	100	0	100	0	100	100	0	100	0
Klebsiella spp. (8)	0	100	37.5	62.5	87.5	12.5	100	0	100	0
Pseudomonas spp. (3)	100	0	100	0	100	0	100	0	100	0
Pseudomonas ATCC 27853	100	0	100	0	100	0	100	0	100	0
Proteus spp. (3)	100	0	100	0	100	0	100	0	100	0

Key: G%=Percentage of growth
I%=Percentage of growth inhibition

Appendix.5. *In vitro* antibacterial activities of crude extract of *Albizia anthelmintica* against clinical isolates & standard Organisms

Clinical Isolates & Standard organisms	**Conc. of extracts (µg/ml)**								**Media & Solvent Control**	
	2000		1000		500		250			
	G%	I%	G%	I%	G%	I%	G%	I%	G%	I%
E.coli (31)	0	100	0	100	0	100	100	0	100	0
E.coli ATCC 25922	0	100	0	100	0	100	100	0	100	0
Klebsiella spp.(8)	0	100	25	75	87.5	12.5	100	0	100	0
Pseudomonas spp. (3)	100	0	100	0	100	0	100	0	100	0
Pseudomonas ATCC 27853	100	0	100	0	100	0	100	0	100	0
Proteus spp. (3)	100	0	100	0	100	0	100	0	100	0

Key: G%=Percentage of growth
%=Percentage of growth inhibition

Appendix.6. *In vitro* antibacterial activities of aqueous fractionated extract of *Albizia gummifera* against clinical isolates & standardorganisms

Clinical Isolates & Standard organisms	**Conc. of aqueous solubilized fraction (µg/ml)**								**Media & Solvent Control**	
	2000		1000		500		250			
	G%	I%	G%	I%	G%	I%	G%	I%	G%	I%
E.coli (31)	0	100	0	100	0	100	100	0	100	0
E.coli ATCC 25922	0	100	0	100	0	100	100	0	100	0
Klebsiella spp.(8)	12.5	87.5	75	25	100	12.5	100	0	100	0
Pseudomonas spp.(3)	100	0	100	0	100	0	100	0	100	0
Pseudomonas ATCC 27853	100	0	100	0	100	0	100	0	100	0
Proteus spp. (3)	100	0	100	0	100	0	100	0	100	0

Key: G%=Percentage of growth
I%=Percentage of growth inhibition

Appendix.7. *In vitro* antibacterial activities of aqueous fractionated extract of *Albizia anthelmintica* against clinical isolates & standard organisms

Clinical Isolates & Standard organisms	**Conc. of aqueous solubilized fraction (µg/ml)**								Media & Solvent Control	
	2000		1000		500		250			
	G%	I%	G%	I%	G%	I%	G%	I%	G%	I%
E.coli (31)	0	100	0	100	51.61	48.38	100	0	100	0
E.coli ATCC 25922	0	100	0	100	0	100	100	0	100	0
Klebsiella spp.(8)	0	100	62.5	37.5	100	12.5	100	0	100	0
Pseudomonas spp.(3)	100	0	100	0	100	0	100	0	100	0
Pseudomonas ATCC 27853	100	0	100	0	100	0	100	0	100	0
Proteus spp. (3)	100	0	100	0	100	0	100	0	100	0

Key: G%=Percentage of growth
I%=Percentage of growth inhibition

Appendix.8. *In vitro* antibacterial activities of n-butanol fractionated extract of *Albizia gummifera* against clinical isolates & standard organisms.

Clinical Isolates & Standard organisms	**Conc. of n-butanol fraction (µg/ml)**										**Media & Solvent Control**	
	2000		1000		500		250		125			
	G%	I%	G%	I%	G%	I%	G%	I%	G%	I%	G%	I%
E.coli (31)	0	100	0	100	0	100	0	100	54.8	45.16	100	0
E.coli ATCC 25922	0	100	0	100	0	100	0	100	0	100	100	0
Klebsiella spp.(8)	0	100	0	100	25	75	75	25	100	0	100	0
Pseudomonas spp.(3)	100	0	100	0	100	0	100	0	100	0	100	0
Pseudomonas ATCC 27853	0	100	100	0	100	0	100	0	100	0	100	0
Proteus spp.(3)	100	0	100	0	100	0	100	0	100	0	100	0

Key: G%=Percentage of growth
I%=Percentage of growth inhibition

Appendix.9. *In vitro* antibacterial activities of n-butanol fractionated extract of *Albizia anthelmintica* against clinical isolates & standard organisms

Clinical Isolates & Standard organisms	**Conc. of n-butanol fraction (µg/ml)**										**Media & Solvent Control**	
	2000		1000		500		250		125			
	G%	I%	G%	I%	G%	I%	G%	I%	G%	I%	G%	I%
E.coli (31)	0	100	0	100	0	100	0	100	38.7	61.29	100	0
E.coli ATCC 25922	0	100	0	100	0	100	0	100	0	100	100	0
Klebsiella spp.(8)	0	100	0	100	0	100	75	25	100	0	100	0
Pseudomonas spp.(3)	33.33	66.66	100	0	100	0	100	0	100	0	100	0
Pseudomonas ATCC 27853	0	100	100	0	100	0	100	0	100	0	100	0
Proteus spp.(3)	100	0	100	0	100	0	100	0	100	0	100	0

Key: G%=Percentage of growth
I%=Percentage of growth inhibition

Appendix .10. *In vitro* susceptibility of clinical & reference strains to standard antibiotics (Tetracycline)

Clinical Isolates & Standard organisms	**Conc. of standard drug (Tetracycline) (µg/ml)**								
	640	320	160	80	40	20	10	5	2.5
	I%	I%	I%	I%	I%	I%	I%	I%	I%
E.coli (31)	100	100	83.87	48.38	35.48	29.03	25.80	25.80	22.58
E.coli ATCC 25922	100	100	100	100	100	100	100	100	100
Klebsiella spp.(8)	100	100	87.5	87.5	75	75	75	62.5	37.5
Pseudomonas spp.(3)	100	100	33.33	33.33	33.33	0	0	0	0
Pseudomonas ATCC 27853	100	100	100	100	100	0	0	0	0
Proteus spp.(3)	100	100	33.33	33.33	33.33	0	0	0	0

Key: I%=Percentage of growth inhibition

Appendix .11. *In vitro* susceptibility of clinical & reference strains to standard antibiotics (Amoxycillin)

Clinical Isolates & Standard organisms	**Conc. of standard drug (Amoxycillin) (µg/ml)**									
	3840	1920	960	480	240	120	60	30	15	7.5
	I%	I%	I%	I%	I%	I%	I%	I%	I%	I%
E.coli (31)	100	67.74	51.61	48.38	45.16	45.16	45.16	45.16	45.16	22.58
E.coli ATCC 25922	100	100	100	100	100	100	100	100	100	100
Klebsiella spp.(8)	87.5	62.5	37.5	37.5	25	25	25	12.5	12.5	12.5
Pseudomonas spp.(3)	100	33.33	0	0	0	0	0	0	0	0
Pseudomonas ATCC 27853	100	100	100	0	0	0	0	0	0	0
Proteus spp.(3)	66.66	66.66	33.33	0	0	0	0	0	0	0

Key: I%=Percentage of growth inhibition

Appendix.12. TLC and chromatogram developed for the plant extracts to show the presence of saponin.