REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

Establishment of urinary tract infections (UTIs), in which an etiological agents and its development of resistance pattern along with the various virulence factors involved in the pathogenesis process for the progression of the disease are discussed in detail in this chapter. In addition, the review of literature is described the currently available and novel antimicrobial strategies for the control of UTI together with the recent advances in biofilm inhibitory activities of the phytocompounds and its inhibitory action.

2.1. Urinary system

The urinary system is a group of organs include the kidneys, urethra, and bladder in the body (Figure 2.1) relevant to filter out excess substances such as fluid and other substances minerals or vitamins as well as blood corpuscles. It is collectively known as urine.

2.2. Functions of the urinary system

One of the major functions of the urinary system is the process of excretion which could eliminate waste products of metabolism and other materials from the body. It also maintains an appropriate fluid volume by regulating the amount of water as well as the concentrations of various electrolytes in the body fluids essential for normal pH of the blood. Among many excretory organs in the body, the kidneys are the most important one. The primary function of the kidneys is to maintain a stable internal environment (homeostasis) for optimal cell and tissue metabolism in resulting of separating urea, mineral salts, toxins, and other waste products from the blood. At least one kidney must function properly for life to be retained.

2.3. Organs of the urinary system

2.3.1. Kidneys and their structure

The kidneys are a pair of bean shaped, reddish brown organs about the size of our fist. It measures 10 to 12 cm long and covered by the renal capsule, which is a tough capsule of fibrous connective tissue. Adhering to the surface of each kidney is of two layers of fat to help cushion them. There is a concaved side of the kidney that has a depression where a juncture of renal artery and a renal vein and lead ureters exit the kidney. The kidneys are located at the rear wall of the abdominal cavity just above the waistline and are protected by the rib cage. They are considered retroperitoneal (lie behind the peritoneum). Three major regions of the kidney are the renal cortex, renal medulla and renal pelvis. The outer, granulated layer is the renal cortex. The cortex stretches down in between a radially striated inner layer. The inner radially striated layer is the renal medulla. This contains pyramid
shaped tissue called the renal pyramids, separated by renal columns. The ureters are continuous with the renal pelvis and are the very center of the kidney (Clapp, 2009).

![Figure 2.1 Anatomy of urinary system in human](http://www.stanfordchildrens.org/content-public/topic/images/01/125801.gif)

2.3.1.1. Role of the kidneys

- **Regulation of plasma ionic composition**: The amount of ions such as sodium, potassium, calcium, magnesium, chloride, bicarbonate and phosphates in body are regulated by the kidney excretes.

- **Regulation of plasma osmolarity**: The kidneys regulate osmolarity because they have direct control over how many ions and how much water a person excretes.

- **Regulation of plasma volume**: They have an important effect on our blood pressure. The kidneys control plasma volume by controlling how much water a person excretes. The plasma volume has a direct effect on the total blood volume, which has a direct effect on our blood pressure. Salt (NaCl₂) causes osmosis subsequently by the diffusion of water into the blood.

- **Regulation of plasma hydrogen ion concentration** (pH): The kidneys partner up with the lungs and they together control the pH. The kidneys have a major role to control the amount of bicarbonate excreted or held on to. The kidneys help to maintain the blood pH mainly by excreting hydrogen ions and reabsorbing bicarbonate ions as needed.
• **Removal of metabolic waste products and foreign substances from the plasma:**
  The most important role is to excrete nitrogenous waste in resulting of splitting amino acid to ammonia. The end product of ammonia is then combined with carbon dioxide to produce urea as the primary nitrogenous end product of metabolism in humans. The liver is then converted the ammonia into urea because it is much less toxic and also excrete some ammonia, creatinine and uric acid. In this mechanism, the creatinine obtained from the metabolic breakdown of creatine phosphate (a high-energy phosphate in muscles) where as uric acid from the breakdown of nucleotides. Uric acid is insoluble and too much uric acid in the blood develops crystals that can collect in the joints and cause gout.

• **Secretion of hormones:** The endocrine system has assistance from the kidneys when releasing hormones such as rennin there by increasing the secretion of aldosterone from the adrenal cortex. Aldosterone promotes the kidneys to reabsorb the sodium (Na⁺) ions. The kidneys also secrete erythropoietin when the blood unable to carry oxygen. Erythropoietin stimulates red blood cell production. The vitamin D from the skin is also activated with help from the kidneys. Calcium (Ca⁺) absorption from the digestive tract is promoted by vitamin D (Reid and Sobel, 1987).

2.3.2. **Ureters**

The ureters are two tubes that drain urine from the kidneys to the bladder. Each ureter is a muscular tube about 10 inches (25 cm) long. Muscles in the walls of the ureters send the urine in small spurts into the bladder. After the urine enters the bladder from the ureters, small folds in the bladder mucosa act like valves preventing backward flow of the urine. The outlet of the bladder is controlled by a sphincter muscle. A full bladder stimulates sensory nerves in the bladder wall that relax the sphincter and allow release of the urine. However, relaxation of the sphincter is also take part in a learned response under voluntary control. The released urine then enters the urethra.

2.3.3. **Urinary bladder**

The urinary bladder is a hollow, muscular and distendible or elastic organ placed on the pelvic floor (superior to the prostate in males), in which anterior border lie on the pubic symphysis as well as its posterior border on the vagina (in females) and rectum (in males). The urinary bladder holds approximately 17 to 18 ounces (500 to 530 ml) of urine despite the desire to micturate is usually about 150 to 200 ml. When the bladder fills with urine (about half full), stretch receptors send nerve impulses to the spinal cord thereby causing it to relax.
and allow the flow of urine into the urethra. The internal urethral sphincter is involuntary. The ureters enter the bladder diagonally from its dorsolateral floor in an area called the trigone. The trigone is a triangular shaped area on the postero-inferior wall of the bladder. The urethra exits at the lowest point of the triangle of the trigone. The urine in the bladder also helps regulate body temperature. If the bladder becomes completely void of fluid, it causes the patient to chill.

2.3.4. Urethra

The urethra is a muscular tube that connects the bladder with the outside of the body. The function of the urethra is to remove urine from the body. It measures about 1.5 inches (3.8 cm) in a woman but up to 8 inches (20 cm) in a man (Kohler et al., 2008). Because the urethra is so much shorter in a woman it makes it much easier for a woman to get harmful bacteria in her bladder this is commonly called a bladder infection or a UTI. The most common bacteria of a UTI are *E.coli* from the large intestines that have been excreted in fecal matter. In female, the urethra is about 1 to 2 inches long and opens in the vulva between the clitoris and the vaginal opening. Therefore women tend to be more susceptible to infections of the bladder (cystitis) and the urinary tract. The urethra in male is about 8 inches long and opens at the end of the head of the penis. The length of a male's urethra, and a number of bends, makes catheterization more difficult. The urethral sphincter is a collective name for the muscles used to control the flow of urine from the urinary bladder. These muscles surround the urethra, so that when they contract, the urethra is closed. The kidneys filter nearly 200 liters of fluid per day from the blood-stream, allowing toxins, metabolic wastes, and excess ions to leave the body in urine while returning needed substances to the blood. As the kidneys perform these excretory functions, they also act as essential regulators of the volume and chemical makeup of the blood, maintaining the proper balance between water and salts and between acids and bases. Frankly, this would be tricky work for a chemical engineer, but the kidneys do it efficiently most of the time.

2.4. Characteristics of urine

2.4.1 Color and transparency

Freshly voided urine is clear and pale to deep yellow. Its yellow color is due to urochrome, a pigment that results from the body's destruction of hemoglobin. It appears like a yellow color when more concentrated. An abnormal color such as pink or brown, or a smoky tinge, followed by eating certain foods (beets, rhubarb) or due to the presence of bile pigments or blood. Additionally, some commonly prescribed drugs and vitamin supplements
alter the color of urine. Cloudy urine may indicate a urinary tract infection (Komala and Sampathkumar, 2013).

2.4.2 Odor

Fresh urine is slightly aromatic, but if allowed to stand, it develops an ammonia odor as bacteria metabolize its urea solutes. Some drugs and vegetables alter the usual odor of urine, as do like some diseases. For example, in uncontrolled diabetes mellitus the urine smells fruity because of its acetone content.

2.4.3 pH

Urine is usually slightly acidic (around pH 6), but changes in body metabolism or diet leads the pH to vary from about 4.5 to 8.0. A predominantly acidic diet that contains large amounts of protein and whole wheat products produces acidic urine. Several factors like vegetarian (alkaline) diet, prolonged vomiting, and bacterial infection of the urinary tract causes the urine to become alkaline.

2.4.4 Specific gravity

Because urine is water plus solutes, a given volume has a greater mass than the same volume of distilled water. The ratio of the mass of a substance to the mass of an equal volume of distilled water is its specific gravity. The specific gravity of distilled water is 1.0 and that of urine ranges from 1.001 to 1.035, depending on its solute concentration.

2.4.5 Chemical composition

The properties of urine include about 95% of water and 5% of solutes (v/v). Apart from these contents, the largest component of urine by weight is urea, which is derived from the normal breakdown of amino acids. Other nitrogenous wastes in urine include uric acid (an end product of nucleic acid metabolism) and creatinine (a metabolite of creatine phosphate, which stores energy for the regeneration of ATP and is found in large amounts in skeletal muscle tissue). Normal other solute constituents of urine, in order of decreasing concentration, are urea, Na, K, PO₄³⁻, SO₄²⁻, creatinine, and uric acid. Much smaller but highly variable amounts of Ca₂⁺, Mg₂⁺, and HCO₃⁻ are also present in urine.

2.5 Urinary tract infection

Reports of Tessema et al. (2007) and Craig et al. (2010) underlines the UTI is one of the most common bacterial infections encountered by clinicians in developing countries and the cause of significant morbidity and mortality among infants and young children, in childhood. It is evaluated that UTI has been affecting approximately 2% of boys and 8% of girls at the age of 7 years (Shaikh et al., 2005). Worldwide studies have indicated that incidence of UTI in children less than 6 years of age ranging from 5% to 17.9% (Reardon et
In view of the National Institute for Health and Clinical Excellence (NICE) guidelines, UTI is defined by a combination of clinical features and the presence of bacteria in urine (NICE, 2007).

Urinary tract infections are categorized into lower tract infection and upper tract infection on the basis of collecting system like urethra and parenchyma (pyelonephritis) (Heffner and Gorelick, 2008). The difference between these two infection systems plays a vital role in accurate diagnosis. Cystitis is defined as an inflammatory condition of the urinary bladder, whereas pyelonephritis as a diffuse pyogenic infection of the pelvis and parenchyma of the kidney. Signs and symptoms of cystitis include dysuria, frequency, urgency, malodorous urine, enuresis, hematuria, and suprapubic pain. Likewise, the signs and symptoms of pyelonephritis include fever over 38.5°C, chills along with costo vertebral angle or flank pain and tenderness with pyuria and positive urine culture (Kirk, 2005). Pyelonephritis represents the most severe type of UTI in children with a great morbidity rate and irreversible damage. UTI symptoms include abdominal pain, back pain, dysuria, frequency, new-onset incontinence, but none of these symptoms alone is sufficient to establish UTI diagnosis in verbal children (Sahi and Carpenter, 2008). The occurrence and clinical manifestation of UTIs is greatly influenced by the gender and age of the child. According to Preda et al. (2007), occurrence of UTI is higher in boys in the neonatal period up to three months of age, but girls overtake them around six months and there is a striking female preponderance beyond infancy. Infections of the urinary system are a significant cause of morbidity in the pediatric population as well (Ross and Pohl, 2008). Approximately 75% of UTIs occur in the first 2 years of life as opined by Ismaili et al. (2011). The first peak of UTI is in the first year of life, and the second peak of UTI occurs between the ages of 2 and 4 years during toilet training. After the age of 6 years, UTIs are infrequent and often associated with dysfunctional elimination (Keren et al., 2008).

### 2.5.1 Urinary tract infection in children

UTIs are serious health affecting problems in worldwide (Bano et al., 2012) and are found to be a common pediatric infection (Islam et al., 2010; Taneja et al., 2010). It occurs in 3 to 5 percent of girls and 1 percent of boys (Staercea et al., 2008). Urinary tract infections may affect 10% of people during childhood with high morbidity and long standing complications like renal scarring, hypertension and chronic renal failure (Salvatore et al., 2011). Early diagnosis and prompt treatments are required to prevent these complications. UTI is one of the common infections in the Indian community. It is well established that the distribution and susceptibility of UTI causing pathogens change according to time and place.
It has also been found that pediatric UTI account for 0.7% of physician office visits and 5 to 14% of emergency department visits by children annually and also associated with high morbidity and long term complications like renal scarring, hypertension, and chronic renal failure. Recent studies on pediatric UTI in India are limited as suggested by Freedman (2005) and Taneja et al. (2010).

Because of differences in anatomy, girls are at a higher risk of UTI than boys beyond the first year of life. In girls, the moist periurethral and vaginal areas promote the growth of uropathogens. The shorter urethral length increases the chance for ascending infection into the urinary tract. Once the uropathogens reaches the bladder, it ascends to the ureters followed by kidneys through undefined mechanism (Chang and Shortliffe, 2006). The urinary tract (i.e., kidney, ureter, bladder, and urethra) is a closed, normally sterile space lined with mucosa composed of epithelium known as transitional cells. The main defense mechanism against UTI is constant ante grade flow of urine from the kidneys to the bladder with intermittent complete emptying of the bladder via the urethra. This washout effect of the urinary flow usually clears the urinary tract of pathogens (Cox and Hinman, 1961). The urine possesses specific antimicrobial characteristics, including low urine pH, polymorphonuclear cells, and Tamm - Horsfall glycoprotein, which inhibits bacterial adherence to the bladder mucosal wall (Sobel, 1997).

UTI occurs as a result of the introduction of pathogens into this space which is associated with adherence to the mucosa of the urinary tract. If uropathogens are cleared inadequately by the washout effect of voiding, then microbial colonization potentially develops followed by microbial multiplication and an associated inflammatory response. Bacteria that cause UTI in healthy hosts often exhibit distinctive properties, known as virulence factors, to overcome the normal defenses of the urinary system (Bower et al., 2005).

In serotypes of *E. coli* frequently isolated in UTI, as they closely adherence to the uroepithelium by their adhesions nature of fimbriae (pili) (Wullt et al., 2000). The interaction of fimbriae with the mucosal receptor triggers internalization of the bacterium into the epithelial cell, which leads to apoptosis, hyper infection, and invasion into surrounding epithelial cells or establishment of a bacterial focus for recurrent UTI (Mulvey et al., 2000; Bower et al., 2005). Uropathogenic strains of *E. coli* have been recognized to release toxins, including cytolethal distending toxin, alpha haemolysin, cytotoxic necrotizing factor-1, secreted auto transporter toxin that causes cellular lysis, cause cell cycle arrest, and promote changes in cellular morphology and function (Uhlen et al., 2000; Guyer et al., 2002; Toth et
To promote survival, various uropathogens possess siderophore systems capable of acquiring iron, an essential bacterial micronutrient, from heme (Russo et al., 2001). Uropathogenic strains of *E. coli* have a defensive mechanism that consists of a glycosylated polysaccharide capsule that interferes with phagocytosis and complement-mediated destruction (Russo et al., 1996).

The infections of the urinary tract affect 2.4% to 2.8% of children every year and account for more than 1.1 million office visits annually. Inpatient hospital costs for children with pyelonephritis total more than $180 million per year in the United States (Freedman, 2005). The epidemiology of pediatric UTI varies based on age and gender. During the first year of life, boys have a higher incidence of UTI; in all other age groups, girls are more prone to developing UTI. During the first year of life, the incidence of UTI in girls is 0.7% as compared to 2.7% in boys (Wettergren et al., 1985). During the first 6 months, uncircumcised boys have a 10 to 12 fold increased risk for developing UTI (Schoen et al., 2000; Wiswell, 2000). In children aged 1 to 5 years, the annual incidence of UTI is 0.9% to 1.4% for girls and 0.1% to 0.2% for boys (Marild and Jodal, 1998). The incidence of a UTI is largely unchanged from age 6 to 16 years, with an annual incidence of 0.7% to 2.3% for girls and 0.04% to 0.2% for boys (Foxman, 2003). During early adulthood (18 to 24 years), the annual incidence of UTI in men remains relatively low at 0.83% (Griebling, 2005). However, it increases substantially in women to 10.8% (Foxman et al., 2000).

The infection is usually caused as a consequence of bacterial invasion of the urinary tract including the lower and the upper urinary tract. Among the bacterial species *Escherichia coli* account to 80% to 85% of the infection followed by *Staphylococcus* species (10% to 15%). The symptoms associated with the bladder and kidney infections are contrasting which includes painful and frequent urination in case of cystitis as a result of bladder infection whereas conditions like high fever and flank pain are commonly experienced in case of kidney contagion which is referred to as pyelonephritis. This prevalence of the infection among children and elderly people is not clearly understood and is currently under study. Bacteria are the prime perpetrator responsible for conferring the infection among humans. Though the infection seems to be harmless in the initial stages, the patient shows a variety of symptoms as the stage progresses and can lead to death in severe circumstances (Vasudevan, 2014).

The diagnosis of UTI requires sampling of the urine for urinalysis and quantitative bacterial culture. It is difficult to obtain uncontaminated urine samples from infants and young children, and the results would lead to false positive diagnosis, over treatment and
unnecessary examinations. Although supra pubic aspiration (SPA) is regarded as the best standard method for urine sampling in infants, but it shows limited success rate (approximately 50%) and invasiveness has restrained its use. Sterile bags and urine pads are considered as reliable to screen infants and young children for possible UTI. As these collection methods involve a significant contamination rate, the final diagnosis should be based on bacterial culture from either a SPA or a catheterization sample (Etoubleau et al., 2009). Urine culture is the gold standard test for the diagnosis and treatment of UTI. However, these methods of obtaining urine from patients and the time required to obtain results of urine culture have resulted in rapid increase the diagnosis as problematic (Quigley, 2009). Urine microscopy with gram stain for the detection of bacteria has become the best single rapid test for UTI diagnosis, but it is expensive, time-consuming and rarely available in primary care. It has been claimed that rapid urine tests are negative in around 10% of children with UTI and thus they should not take the place of urine culture (Williams et al., 2010). UTI has a potential for serious and life-threatening sequelae as there is no availability of timely and appropriate medical intervention due to inadequate numbers of health care providers.

2.6. Uropathogens

Research studies have defined urinary tract infection as the most common form of bacterial infection (Parveen et al., 2011; Demilie et al., 2012). Escherichia coli are the predominant facultative anaerobe of the human colonic flora. The organism typically colonizes the infant gastrointestinal tract within hours of life and thereafter, E. coli and the host derive mutual benefit (Drasar and Hill, 1974). It has been traditionally described that certain serotypes of E.coli are consistently associated with uropathogenecity and were designated as uropathogenic E.coli (UPEC). One of the most important, especially in the case of E. coli, is the ability of these bacteria to adhere to the mucous membranes in the urinary tract. The mucous membranes of the lower urinary tract contain a variety of molecules, including mannose, a sugar. Strains of E. coli can adhere to these mannose molecules using small projections, called fimbriae (Ermel et al., 2012). This binding prevents bacteria from being cleared from the urinary tract by the flow of urine, which is normally a deterrent to bacterial colonization (Mulvey, 2002). Once the bacteria have bound to the cells that line the urinary tract helps invade these cells and promote themselves to resistant against antibiotics or the immune system (Dhakal et al., 2008; Jorgensen and Seed, 2012).

Uropathogens typically gain access to the urinary system through ascending migration through the urethra from the vaginal introitus contaminated by fecal flora. The most common pathogen in uncomplicated UTI in women is E.coli, which accounts for approximately 80 %
of total cases. Moreno et al. (2006) also evidenced a specific strains of *E. coli* have commonly been identified as pathogenic to human beings.

2.7. Uropathogenic properties of uropathogens

Among bacterial isolates, *E. coli* is found to be the leading cause of UTI (80 to 85%). Biofilm formation and haemolysin production by Uropathogenic *E. coli* (UPEC) have been known virulence factors to cause UTI. It is detected that, there was no correlation between haemolysin production and biofilm formation. Therefore the treatment of UTIs is emerging difficult as it bearers of Multi Drug Resistant (MDR) bacterial pathogens (Nair et al., 2013). Biofilm may form on variety of surface, including living tissue, indwelling medical devices and water pipes, etc. Biofilm has a tolerance to protect the bacterium from host defense mechanisms and antibiotic action. Bacterial biofilms are often associated with long term persistence of bacteria in various environments exhibits increased resistance to antibiotic (Soto et al., 2007; Sharma et al., 2009). Fimbriae are also known as one of the factors which contribute to form biofilm on the surface. Various structures such as flagella, fimbriae, outer membrane proteins, curli and extra cellular polymeric matrix are found to involve in biofilm formation (Pruss et al., 2006).

UPEC utilizes P fimbriae to bind specifically to the P blood group antigen which contains a D galactose D galactose residue. Binding of this P fimbriae is not only specific to red blood cell but also to a specific galactose disaccharide that is found on the surface of uroepithelial cells in approximately 99% of the population. Literature of Akram et al. (2007) have shown that UTI could associates in both upper and lower tract. Of these, lower tract UTI described as cystitis. The major symptoms of cystitis are the urgency of urination, dysuria, irritation of urinary tract and tiredness. Most of the cases UTI occur as community acquired infection.

Abuse and improper prescribing policy of antibiotics causes remarkable increase of antibiotic resistance pattern among the *E. coli* isolates from UTI (Li et al., 2007). Microorganisms seem to MDR by exhibiting a resistance to at least three antibiotics (Santo et al., 2007). Frequency of UTI cases caused by MDR *E. coli* required strong concern of medical practitioners and health agencies. Therefore regional studies on pattern of antibiotic sensitivity could offer a solution to overcome this problem (Dash et al., 2012).

2.7.1. Virulence factors in *E. coli*

Most UTIs in newborns are caused by gram negative aerobic bacilli, *E. coli*, which is a significant increase in the newborn period, accounting for up to 80 % of UTIs in most large series (Tamim et al., 2003). *E. coli* is the most common cause of uncomplicated urinary tract
infections and accounts for approximately 75 – 95 % of all infections (Nicolle, 2003). Zhao et al. (2009) have explained that UTI is one of the most prevalent bacterial infections, therefore increasing the financial burden on family and society is substantially. UPEC are the most common microorganisms causing UTIs. UPEC strains possess specialized virulence factors, enabling them to colonize and invade to the host followed by disrupt the host defense mechanisms and injuring to host tissues and /or stimulate a noxious host inflammatory response. Virulence factors of recognized importance in the pathogenesis of UTI include diverse adhesions (eg., P, S and F1 C fimbriae and type 1 fimbriae), toxins, siderophores and polysaccharide coatings. However, only a very few epidemiologic data about the virulence factors of UPEC are available. Because UPEC yearly affects a large proportion of the population, they are a major target of antimicrobial therapy. The resistance rate to nalidixic acid and ampicillin was found to be greater than 90%. Perrotta et al. (2008) stated that, in post menopausal women, vaginal estrogens reduce the number of recurrent UTIs by decreasing the vaginal pH and permitting a vaginal flora in which uropathogens are less likely to dominate.

2.7.1.1. Adherence to host cells

Adhesion to host cell surfaces is considered as the primary step of uropathogens in facilitating UTIs (Bouguenec et al., 1992; Holden et al., 2006). Such attachments brought about several important advantages for bacterial survival, including the prevention of being swept out by the normal flow of host body fluids such as urine and the establishment of Intracellular Bacterial Communities (IBCs) for further maturation and multiplication (Blanco et al., 1997). The Virulence Factors (VFs) responsible for uroepithelial cell adherence are given by a variety of adhesion and fimbriae classes. Fimbriae can be classified as to whether they could facilitate erythrocyte agglutinations in the presence of mannose. Adhesins whose agglutinations were unaffected by mannose were regarded as mannose resistant as in the case of mannose resistant adherence (MRHA). Alternatively, mannose sensitive adherence (MSHA) represented those adhesions in which cell attachments could be inhibited by mannose (Johnson, 1991). The expressions of these various types of fimbrial and nonfimbrial adhesins have been suggested as common in E. coli isolates (Silveira et al., 2001).

2.7.1.2. Mannose resistant adhesions (P fimbriae)

P fimbriae are found to exhibit MRHA by binding to the P blood group antigens of human erythrocytes as inferred by O’Hanley et al. (1983), Johnson et al. (2001a) and Marrs et al. (2005). P blood group antigens referred to an oligosaccharide family composed of a terminal or internal Gal (α1 4) Gal (Gal-Gal) moiety (Johnson et al., 2000b). The Gal-Gal
moieties are present on particular mammalian cells only as glycosphingolipids and carbohydrate components (Marcus et al., 1981; Johnson, 1991). Glycolipids with Gal-Gal moieties are found to be one of the receptors for E. coli adherence. In E. coli strains that recognize P blood group antigens, all Gal-Gal containing glycolipids suitable receptors for adherence but not the glycolipids without Gal-Gal moieties (Svenson et al., 1983; Bock et al., 1985). The binding specificities for every P fimbriae E. coli, however, are not identical. Some of the strains facilitated lower adherence to both globotriasoylceramide and globoside. Furthermore, the Gal-Gal moiety might be required for the adherence of some other strains (De Man et al., 1987). P fimbriae receptors have been found in human erythrocytes, uroepithelial cells, and polymorphonuclear leukocytes (PMNLs). However, the densities of Gal-Gal containing glycolipids are different. The Gal-Gal containing glycolipids found in human erythrocytes and uroepithelial cells are more abundant than those present in PMNLs, thus facilitating the binding of E. coli cells to trigger UTIs while minimizing the chances of phagocytosis due to accidental adherence to PMNLs (Macher et al., 1980; Johnson, 1991).

2.7.1.3. S fimbriae and F1C fimbriae

S fimbriae and F1C fimbriae are closely related to VF s but possess diverse binding specificity patterns. S fimbriae are specifically bound to terminal sialyl-galactosidase residues of human erythrocytes in order to facilitate MRHA (Parkkinen et al., 1983; Parkkinen et al., 1989). E. coli strains with S fimbriae are strongly associated with bacteremia as well as with meningitis rather than with UTIs (Ott et al., 1986). The F1C fimbriae, however, are found to bind to renal cells and buccal epithelial cells (Johnson, 1991). A number of regions in the human body are found to exhibit the binding sites of S fimbriae, especially in the Bowman’s capsule, glomerulus, and the connective tissue of the bladder (Emody et al., 2003). F1C fimbriae, however, binded only to a narrow spectrum of locations including the vessel walls of the kidneys and bladder; they also primarily targeted endothelial cells as evidenced by Johnson (1991).

2.7.1.4. Dr Family of adhesins

The Dr Blood group antigen, which is a decay accelerating factor, is found to be the common receptor of the Dr Family of adhesins (Arthur et al., 1989; Nowicki et al., 1990). A number of members could be involved in this family such as afimbrial adhesins I (AFA-I), AFA III, and O75X adhesins (Larbigne-Roussel et al., 1988). Differences in the structural morphology are observed among the Dr Family of adhesins and the other adhesin types. The coil-like structure and the fine mesh of filamentous coating conformations might enhance the adherence to particular binding sites. The receptors of the Dr Family of adhesins are mainly
focused at kidney and bladder tissues. Bowman’s capsule, proximal, and distal tubules as well as bladder connective tissues are found to exhibit a high abundance of receptors. The Dr Family of adhesins is especially associated with cystitis in which about 26 to 50% of patients suffering from such acquire Dr-related sequences. The prevalence was higher than that in pyelonephritis and ABU which accounted for 26% and 6%, respectively (Johnson, 1991). The prevalence of Dr Family adhesins was higher in cystitis isolates than in fecal isolates (Marrs et al., 2005).

2.7.1.5. Mannose sensitive adhesions - Type 1 fimbriae

As D- mannose or α-methyl mannoside could facilitate the blocking of type1 fimbriae in host cells, it was proposed that mannose residues and mannose-containing glycoprotein were indispensable components which were a prerequisite to type1 fimbriae adherence (Ofek et al., 1977; Salit et al., 1977; Mulvey et al., 2000). In addition to mannose residues, type1 fimbriae binding could also be inhibited by nitrophenol and its derivatives. Diverse putative receptor structures were found to be involved in the type1 fimbriae binding process. Erythrocytes were found to be the main reservoir of type1 fimbriae. In addition, vaginal cells, intestinal cells and epithelial cells in the buccal cavity could also mediate type1 fimbrial adherence. In the urinary tract, the binding sites of type1 fimbriae were concentrated mainly at the muscular layer of the bladder, proximal tubulus, and the vascular connective tissue layers of vessels in the kidneys (Johnson, 1991).

2.7.2. Cytotoxicity

2.7.2.1. Haemolysin

The most common type of haemolysin produced by E. coli is alpha haemolysin, which causes the lysis of erythrocytes of all mammals (Cavaleri et al., 1984). Calcium is required for the haemolysis process, by which the modification of haemolysin by calcium makes to haemolytically competent (Rennie et al., 1974). The haemolysis process is first triggered by inserting haemolysin into a lipid containing cytoplasmic membrane. It also enhanced the permeability to Ca$^{2+}$, K$^+$, and sucrose as well as mannitol of erythrocytes. The imbalance of ion potentials and concentrations influenced in the equilibrium and normal function of erythrocytes results the lysis of erythrocytes occurred (Johnson, 1991). Haemolysin was also toxic to PMNLs and could exert cytotoxic effects. At lower concentrations, haemolysin could facilitate degranulation, leukotriene releases; disrupt chemotaxis and phagocytosis. Meanwhile, cell lysis could be triggered under high haemolysin concentrations (Gadeberg et al., 1984; Scheffer et al., 1988). Different tolerance levels have
been observed among various immune cells to haemolysin exposure. Gadaberg et al. (1984) portrayed lymphocytes to be more resistant to haemolysin than granulocytes and monocytes.

2.7.2.2. Extended Spectrum β Lactamse (ESBL) production

One of the most important resistant mechanisms in gram negative bacteria against β lactum antibiotics is induced by the production of β lactamase enzymes, which are classified in to 4 major groups including A, B, C and D based on their inhibition mechanism, type of substrate and physical characterization such as molecular weight and isoelectric point. The gram negative bacteria have rapidly expanded resistance to broad spectrum β lactum antibiotics during the past two decades (Nazemi et al., 2012). Shahcheraghi et al. (2008) reported that, more than 200 types of ESBL have been found world wide, most of them belonging to the Enterobacteriaceae family. The incidence of community acquired UTIs due to ESBL producing E.coli has increased worldwide (Rodriguez – Bano et al., 2008).

2.7.2.3. Cytolethal distending toxin (CDT)

The bacterial VF toxin CDT exerted its cytotoxic effects mainly by means of cell cycle arrest. It irreversibly blocked cells growth in either the G1 or G2 phase of the cell cycle by halting the cell maturation and cell division process (Scott et al., 1994). Cell cycle arrest is a consequence of the direct DNA damage of the CdtB protein, which belonged to a class of phosphodiesterases including nucleases. Pickett et al. (2004) also went on to report CDT could contribute to distension of cell lines and hence, cell death.

2.7.2.4. Cytotoxic necrotizing factor (CNF)

CNF1, one of the CNF members, is another common toxin produced by E. coli causing UTIs. A variety of properties that facilitate pathogenesis have been reported about CNF1. First, it contributed to type 3 complement receptor (CR3) dependent phagocytosis reduction in monocytes (Rippere-Lampe et al., 2001). It enhances the flushing effect of brush border and inhibits PMNL transmigration. The wound repair processes of bladder and fibroblast cells are also affected. In the urinary system, the apoptosis of bladder cells have been reported under subsequent CNF exposure. The CNF-positive E. coli strains facilitated deep and more severe inflammation since the presence of CNF reduced the immune responses posed by neutrophils, thus allowing these strains to infiltrate into deeper tissue to trigger UTIs. The synthesis of CNF1 has been encoded by the cnf1 gene, which has found in chromosome or pathogenicity islands (Rippere-Lampe et al., 2001).

2.7.3. Serum resistance

Capsule polysaccharide could facilitate enhanced survival in the serum of the strains. The serum resistance among the encapsulated strains is dependent on the capsular types and
capsular material proportion. Some capsular types such as K1 polysaccharide encapsulated strains have been found to be associated with an increase in serum resistance. However, other factors or determinants might also be involved in the enhancement of serum resistance as evidenced by Johnson (1991).

In general, \textit{E.coli} possesses 80 types of capsule polysaccharides like linear polymers with repeating carbohydrate residues. In addition, lipids or amino acid components are also found as the next major constituents in the capsule polysaccharide structure (Johnson, 2003a). The K antigens present in different capsules of \textit{E. coli} were various and thus the capsules could be classified into different capsular types. The K capsular type identification could be carried out by K- specific phages and antiserum. Several K capsular types have been identified in \textit{E. coli} such as K1, K5, K12, and K13. Of these, the K1 capsule associated with UTIs and considered as the most commonly identified capsular type.

\textbf{2.7.4. Serotyping of uropathogens}

The O antigen, which contains many repeats of oligosaccharide units, is a part of the lipo polysaccharide present in the outer membrane of gram negative bacteria. The O unit is synthesized by sequential transfer of a sugar phosphate and sugars from respective nucleotide sugars to the carrier lipid, undecaprenyl phosphate (Undp). O units are then polymerized on Undp in to polysacharide chains, which are transferred to the independently synthesized core lipid A to form LPS (Reeves, 1994). Characteristically, all genes specific to O antigen synthesis in \textit{E.coli} are clustered between the \textit{galF} and \textit{gnd} genes (Revees \textit{et al.}, 1996.)

The O antigen contributes major antigenic variability to the cell surface and 166 O antigen forms have been recognized in \textit{E.coli}. Accordingly the surface O antigen is subject to intense selection by the host immune system, which may account for the maintenance of many different O antigen forms with in the species such as \textit{E.coli} (Wang \textit{et al.}, 2001).

\textbf{2.7.5. Iron acquisition – Virulent factors}

\textbf{2.7.5.1. Aerobactin}

Iron is an essential element in bacterial growth as well as in a variety of metabolic pathways, including oxygen storage and transport, peroxide metabolism and DNA synthesis. Due to the low iron concentrations of diluted urine and complement-depleted serum, \textit{E. coli} utilizes siderophores for the enhancement of iron acquisition (Neilands \textit{et al.}, 1985; Johnson \textit{et al.}, 1988; Naveen \textit{et al.}, 2005). One of the bacterial siderophores, aerobactin, is commonly found in \textit{E. coli} isolates. It was reported that aerobactin is produced by condensation process involving two lysine and one citrate molecule (Slavchev \textit{et al.}, 2009). A multi-step pathway is involved in iron sequestration by aerobactin. The extraction of Fe$^{3+}$ iron from host iron-
binding proteins is carried out by a 74 kDa outer membrane receptor protein followed by the bacterial cells for further utilization and metabolic reactions.

2.7.5.2. Other siderophores components

It has been found that some other iron acquisition systems are also present in E. coli followed by Enterobactin. However, several differences have also been found between enterobactin and aerobactin. The solubility of enterobactin is lower than that of aerobactin. In addition, enterobactin released free iron to the cytoplasm, whereas aerobactin transported iron directly to bacterial iron centers. Salmonchelin and Yersinia siderophore receptor are encoded by the iroN and fyuA gene could also be identified in E. coli (Russo et al., 2002). This indicated that one iron uptake system could be shared by different species (Johnson et al., 2000c; Hejnova et al., 2005).

2.8. Pathogenesis of UTI

2.8.1. Attachment of uropathogens to the host epithelium

A multi-step pathogenesis pathway includes the binding of the E. coli bacterial cells to the host uroepithelium and transmitted from the urethra to the host bladder. After 4 to 24 hours of infections, selective pressure by the host bladder triggered the expression of a VF in E. coli, the type I fimbriae (Kaper et al., 2004). A constitutive expression of type I fimbriae is observed in cystitis cases when the infection targeted the host bladder. However, in pyelonephritis strains, an invertible element is present in which type 1 fimbriae expression is suppressed and less expressed (Kaper et al., 2004). The type 1 fimbriae facilitated tight bindings between E. coli and the uroepithelial cells, thus preventing the physical flushing out effect of urine flow.

2.8.2. Intracellular multiplication of uropathogens in the host bladder

As the infection proceeded, a complex cascade is activated and formed IBCs as observed by Kau et al. (2005). Initially, the reversible then later the irreversible attachments were facilitated between the bacterial cells and the surface of the uroepithelium with the aid of extracellular polymeric substances. A loose collection of fast growing and rod-shaped E. coli then changed into slow-growing and well-structured biofilm-like communities. The communities occupied most of the cytoplasm of the umbrella cells, contributing to luminal protrusions and pod formations. The IBCs are established such that E.coli organized cooperatively to resist host immune responses and to spread on the bladder surfaces. Further IBC formations caused IBCs to express motile rod-shaped phenotypes. Then the E. coli at the IBC periphery started to detach from the uroepithelial cells. The fluxing out of E. coli continued to spread and to infect the surrounding cells, resulting in cystitis (Kau et al., 2005).
2.8.3. Impact of uropathogenic strain on kidney’s function

In strains causing pyelonephritis, the *E. coli* approached the host kidneys through the ureters. The *E. coli* then colonized at the tubular epithelium, and expressed several VFs such as the P fimbriae. In serious UTIs and immunocompromised UTI cases, the *E. coli* leave the kidneys by breaking down the host barrier followed by the bacterial cells travelled through the bloodstream to the other organs of the host, there by mediating bacteraemia to the host (Kau *et al.*, 2005).

2.8.4. Host responses to uropathogens

Different host responses have been observed between the colonization of *E. coli* causing UTIs and commensal *E. coli* due to the lack of virulence-associated adhesive ligands. As a result, the binding to signal receptors on the mucosal cell surfaces of the host by commensal *E. coli* is unavailable as illustrated by the mouse model. However, the UTI producing *E. coli* possessed adhesins which promoted host cell attachment. Thus, cell adherence is to be an indispensable step in activating the signaling cascades of the host immune system (Svanborg *et al.*, 2006).

2.8.5. Apoptosis and cell exfoliation

Attaching *E. coli* to the host uroepithelium facilitated apoptosis and exfoliation. The programmed cell death and cell shedding aimed at removing infected outermost uroepithelial layers together with the *E. coli* bacterial cells out of the host. The exfoliation of the superficial facet cells started within hours after infection. It involved transcriptional regulation as well as differentiation and proliferation of uroepithelial cells. Cell exfoliation also led to pro-inflammatory response (Svanborg *et al.*, 2006). The superficial umbrella cell differentiation and division have been quiescent in healthy individuals, whereas sharp increases observed in cell proliferation rates in UTI patients (Svanborg *et al.*, 2006).

2.8.6. Inflammatory responses caused by P fimbriae-mediated binding

Several VFs and lipopolysaccharides (LPS) could contribute to inflammation. Of these, the P fimbriae are one of the most important. Once P fimbriae adhered to receptor epitopes in the globoseries of glycosphingolipids (GSLs) on the uroepithelial cells activated the Toll-like receptor 4 (TLR4) adaptor proteins TRAM/TRIF. The chemokine CXCL8 is then secreted which stimulated the influx of neutrophils to cross the uroepithelium. For patients with mutated or defective TLR4, the initiation of immune responses is absent, thereby contributing to ABU. However, the UTI causing *E. coli* possessed adhesins which promoted cell adherence to host is an indispensable step in activating the signaling cascades of the host immune system (Svanborg *et al.*, 2006).
2.9. Antibiotic susceptibility pattern of uropathogens

UTI very often treated with broad spectrum of antibiotics where as one narrower spectrum antibiotic is found to be effective. The derivatives of fluoroquinolone are commonly preferred as initial agents for empiric therapy of UTI because of their high bacteriological and clinical cure rates. Abuse and improper prescribing policy of antibiotics causes remarkable increase of antibiotic resistance pattern among the E. coli isolates from UTI (Li et al., 2007). In light of the high UTI incidence rates, medical diagnosis and treatment expenditures posed prominent and heavy financial burdens to governments around the world. It has been estimated that 2.4 billion dollars spent in curing UTI patients in the United States in the year 2000 (Lane et al., 2005). They also suggest that, the evolutionary changes of pathogens and mostly, the emergence of multidrug-resistant strains is occurred during UTI treatments.

In most of developing countries, these organisms have shown increased drug resistance over the last two decades in resulting the management of these patients would become a major problem. A continuous monitoring of bacterial epidemiology and antibiotic susceptibility has been a practice of crucial for clinicians in choosing appropriate empirical antibiotic for the treatment of UTI before obtaining the microbiological results (Movahedian et al., 2007). Subsequently, increasing antimicrobial resistance among uropathogens poses a challenge to therapy and the pattern of this resistance varies geographically. Fluoroquinolones have become popular treatments for patients with uncomplicated UTI because of E. coli’s emerging resistance to other common medications (Mehnert – Kay, 2005). UTI due to MDR E. coli increases the cost of treatment, morbidity and mortality especially in developing countries like India as reported by Aljiffri et al. (2011).

Joshi (2010) stated that, the hospital antibiogram is a periodic summary of antimicrobial susceptibilities of local bacterial isolates submitted to the Clinical Microbiology Laboratory of hospitals. Antiogram are often used by the clinicians to assess local susceptibility rates, as an aid in selecting empiric antibiotic therapy and in monitoring resistance trends over time with in an institution. WHONET (World Health Organization NET) is free Windows – based database software developed for the management and analysis of Microbiology laboratory data, in order to a special focus on the analysis of antimicrobial susceptibility test results.

Micro organisms considered multidrug resistance (MDR) when develop resistance to at least three antibiotics (Santo et al., 2007). Frequency of UTI cases caused by multidrug resistance E. coli required strong concern of medical practitioners and health agencies.
Therefore regional studies on pattern of antibiotic sensitivity are very much necessary to overcome this problem. From the last few years antibiotic resistance property of pathogenic *E. coli* has become a serious threat for human health. Multi drug resistance pattern turns a great challenge for medical practitioners to formulate antibiotic prescribing policies (Dash *et al.*, 2012).

### 2.10. Plant materials as phytotherapy

The plant kingdom represents an enormous reservoir of biologically active molecules. Phytochemical investigation of higher plants with known ethno botanical information has attracted the attention of researchers as diverse biological activities of plant extracts and phytochemicals are being reported. Phytochemicals have higher commercial value in the local and global markets because of shifting from illness oriented products to wellness promoting products. Besides that prevalence of chronic diseases that cannot be cured by conventional drugs, make herbal based phytochemical industry is an upcoming industrial sector.

According to World Health Organization, medicinal plants serve as the best source for a variety of drugs. Therefore, such plants are being investigated for better understanding of their medicinal properties (Alo *et al.*, 2012). Plants have been used since ancient times to treat common infectious diseases whether locally within the dermis or a blood infection (Birgul *et al.*, 2009). Resistance towards drugs developed by pathogenic microorganisms due to their indiscriminate use and side effects due to synthetic drugs has recently drawn much attention towards plant extracts and biologically active compounds isolated from plant species used in herbal medicine. Medicinal plants represent an alternate to synthetic drugs for the treatment of several non severe infectious diseases and can also be a possible source for new potent antibiotics to which pathogen strains are not resistant (Mathur *et al.*, 2011). Plants play an important source of potentially useful structures for the developments of new chemotherapeutic agents (Mahesh and Satish, 2008). In resulting of above discussion, medicinal plants have attracted the attention of several biological communities (Penecillia and Magno, 2011).

Plants produce wide array of bioactive compounds/molecules or phytochemicals which probably evolved as chemical defense against predation or infection but, are now found to be useful for treatment of various ailments. Due to the myriad of potential benefits they possess, plants have been widely exploited in traditional medicine and their curative potentials are well documented (Krishnaiah *et al.*, 2009). These antibacterial active principles isolated from higher plants appear to be one of the important alternative approaches to
contain antibiotic resistance and in the management of disease. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects (Satish et al., 2008). Plants have been used since time immemorial for their antimicrobial traits shown by the various secondary metabolites synthesized and deposited in specific parts or all parts of plant. Screening of these compounds and identification of the bioactive molecules and their antimicrobial properties are the needs of the hour. Methanol has been found to be the most effective solvent enabling maximum separation of the different phytochemicals, and preliminary analysis of the extracts which revealed the presence of flavonoids, phenols, saponins tannins in both fruit as well as extract of E. officinalis (Javale and Sabnis, 2010).

The use of traditional medicinal plants for primary health care has steadily increased worldwide in recent years. Currently, out of 80% of pharmaceuticals derived from plants, very few are now being used as anti microbial. Plants are rich in a wide variety of secondary metabolites that possess anti microbial properties (Samy and Gopalakrishnakone, 2008). The traditional plants may represent new sources of antimicrobial with stable, biologically active components could establish a scientific base for the use of plants in modern medicine. The global documentation of 85,000 plants in medicinal use signifies the interest of scientists and medical professionals for their remedies as well as recognition for true benefits. A single plant contains a large number of bioactive compounds, indicating their potential as a source of new drugs (Kathirvel and Sujatha, 2012).

Consumption of various types of fruit provides excellent health benefits as their phytochemicals content prevent the diseases. Epidemiological studies have revealed that there is a positive association between the intake of both vegetables and fruits and a reduction of various diseases (Ikram et al., 2009). Fruits contain many different kinds of antioxidant compounds, including flavonoids, phenolics, carotenoids and vitamins which are all considered beneficial to human health, for decreasing the risk of degenerative diseases by reduction of oxidative stress and for the inhibition of macromolecular oxidation (Rangkadilok et al., 2007).

There is a huge biodiversity in India including varied geography, climatic changes and absence of standard practice of cultivation thereby leading to variations in composition and concentration of phytoconstituents of plant materials. Adulterations and contaminations are regular events in herbal drug manufacturing. Therefore, it is essential to ensure that raw materials used in the manufacturing of the drugs are not only authentic but also of prescribed quality and hence identification and evaluation of raw materials have become fundamental
need of herbal industry. In resulting of this, the fingerprinting and marker compound analysis by chemical and validated chromatographic techniques have been gaining more importance for use in standardizing herbal medicinal formulations. Plants have been used for their antimicrobial traits owing to its ability in producing various secondary metabolites synthesized and deposited in specific parts or all parts of plant. Screening of these compounds and identification of the bioactive molecules and their antimicrobial properties are in needs of the hour. Medicinal plants have attracted the attention of several biological communities (Penecillia and Magno, 2011). In lighting of this background, five plants such as *Allium sativum*, *Coriandrum sativum*, *Curcuma longa*, *Emblica officinalis* and *Terminalia chebula* were selected on the basis of ethanobotanical and ethanopharmaceutical literature for the present research study and its identified characters were deployed in Table 2.1.

2.10.1. *Allium sativum*

*A. sativum*, commonly known as garlic has been used widely in food and medicine over 5000 years like other herbs and spices (Karuppaiah and Rajaram, 2012). It is a species of monocot, bulb-forming perennial. Garlic bulbs contain separate fleshy sections (cubes) shown in Figure 2.2 and each covered with a papery skin (tunic). The plants produce a leafless flower stem (a scape), but the flowers are sterile and produce bulbils (small cubes) rather than seeds; the species is propagated clonally from cloves and bulbils (Block, 2010). Garlic (*Allium sativum* L.) is also cultivated all over India and is used as a vegetable. The traditional Ayurvedic healers consider garlic as an excellent natural product that has immense therapeutic potential in many pathological conditions.

Garlic has been used in various forms such as oil, powder, raw juice extracts and possesses many therapeutic properties including antimicrobial, antineoplastic, antcardiovascular, immuno-stimulatory and hypoglycaemic activities. Its antimicrobial activity is attributed to its key component allicin, which is rapidly synthesised from its precursor when garlic is crushed (Jabar and Ai – Moossawi, 2007). The therapeutic effect of garlic has been attributed to its organosulfur constituents, which are responsible for its typical flavour and odour (Zeng *et al.*, 2012).
<table>
<thead>
<tr>
<th>S.No</th>
<th>Botanical Name</th>
<th>Family</th>
<th>Common name (in Tamil)</th>
<th>Used plant parts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Allium sativum</em></td>
<td><em>Amaryllidaceae</em></td>
<td>Garlic (Vellai poondu)</td>
<td>Clove</td>
<td>Gaherwal <em>et al.</em> (2014)</td>
</tr>
<tr>
<td>2.</td>
<td><em>Coriandrum sativum</em></td>
<td><em>Apiaceae</em></td>
<td>Coriander (Malli)</td>
<td>seeds</td>
<td>Rathabai and Kanimozhi (2012)</td>
</tr>
<tr>
<td>3.</td>
<td><em>Curcuma Longa</em></td>
<td><em>Zingiberaceae</em></td>
<td>Turmeric (Manjal)</td>
<td>Rhizome</td>
<td>Saleem <em>et al.</em> (2011)</td>
</tr>
<tr>
<td>4.</td>
<td><em>Emblica officinalis</em></td>
<td><em>Euphorbiaceae</em></td>
<td>Amla (Nellikkai)</td>
<td>fruit</td>
<td>Varghese <em>et al.</em> (2013)</td>
</tr>
<tr>
<td>5.</td>
<td><em>Terminalia chebula</em></td>
<td><em>Combretaceae</em></td>
<td>Myrobalan (Kadukkai)</td>
<td>Dried fruit</td>
<td>Bag <em>et al.</em> (2012)</td>
</tr>
</tbody>
</table>
2.10.1.1. Taxonomical classification of *A. sativum*

<table>
<thead>
<tr>
<th>Classification</th>
<th>Taxonomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domain</td>
<td>Eukarya</td>
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<tr>
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<td>Plantae</td>
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<tr>
<td>Phylum</td>
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<td>Lilopsida</td>
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<td>Allium</td>
</tr>
<tr>
<td>Species</td>
<td>Allium sativum</td>
</tr>
</tbody>
</table>

2.10.1.2. Pharmacological activities of *A. sativum*

Leaves and cloves of *A. sativum* have been used in traditional medicine of Iran and other countries for a long time (EL-Mahmood, 2009; Mikaili and Mehdioghli, 2010). Allicin and other sulfur compounds are considered to be the major compounds responsible for the antimicrobial effect of garlic. Garlic is effective against a number of gram-negative, gram-positive and acid-fast bacteria, including *Staphylococcus, Salmonella, Vibrio, Mycobacteria,* and *Proteus* species (Tariq *et al.*, 1988). In a study by Lai and Roy (2004), fresh extracts of *A. sativum* (garlic) and *Nigella sativum* (black cumin) have the properties of more antibacterial activity against the isolates of the UTI, compared to the individual extract or drugs, such as cefalexin, cotrimoxazole, and nalidixic acid. Allicin from garlic possess antifungal activity particularly against *Candida albicans* (Ankri and Mirelman, 1999). The antiviral activity of garlic in humans may be secondary to a direct toxic effect on viruses. It also enhanced NK-cell activity that destroys virus infected cells (Tariq *et al.*, 1988). Another study indicated that Allicin has antiparasitic activity against *Plasmodium falciparum* and *Trypanosoma brucei* (Waag *et al.*, 2010). Alcoholic extracts of *A. sativum* also have anti-cryptococcal activity against murine disseminated cryptococcosis (Khan and Katiyar, 2000). Garlic extract is found to have a superior immune modulatory property over raw garlic extract (Chandrashekar and Venkatesh, 2012). It produces various sulfur compounds along with their breakdown products and yield a characteristic pungent taste and odor, which may persist on the breath and body for up to 30 hours (Block, 2010). These all compounds of garlic have documented as antimicrobial and antifungal agents in reducing fungal infections and parasites, lowers blood cholesterol, treats arterosclerosis, and promotes circulatory function (Block, 2010).
2.10.2. *Coriandrum sativum*

Coriander is considered as annual popular culinary medicinal plant with a distinctive pungent, fatty, and aldehydic aroma, which has been cultivated since ancient times. It is originally originated from the Mediterranean and Middle Eastern region and grows extensively in India, Russia, Central Europe, Asia and Morocco. It grows up to 25 to 60 cm (9 to 24 in.) in height with thin, spindle-shaped roots, erect stalk, alternate leaves and small, pinkish-white flowers. The plant flowers from June to July and yields round fruits consisting of two pericarps. The plant is cultivated for its aromatic leaves and seeds (Burdock and Carabin, 2009). The seeds of coriander (Figure 2.3) were found in the ancient Egyptian tomb of Ramses the Second. It has been used for cooking and for children’s’ digestive upset and diarrhoea. Coriander is recognized as one of the most important spices in the world due to its great significance in international trade. The seeds because of its extensive medical applications, it has been traditionally applied for curing digesting disorder's pain in joints and rheumatism, stomachic, spasmylytic, carminative, diarrhoea and dyspepsia of various origins and also used in aromatherapy.

2.10.2.1. Taxonomical classification of *C.sativum*

<table>
<thead>
<tr>
<th>Kingdom</th>
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</tr>
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<tr>
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<td>Magnoliopsida</td>
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<tr>
<td>Super order</td>
<td>Asteranae</td>
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<td>Order</td>
<td>Apiales</td>
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<tr>
<td>Family</td>
<td>Apiaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Coriandrum</td>
</tr>
<tr>
<td>Species</td>
<td>Coriandrum sativum L.</td>
</tr>
</tbody>
</table>

2.10.2.2. Pharmacological activities of *Coriandrum sativum*

Increased antimicrobial potential of aqueous infusions and aqueous decoctions of *E.officinalis* and *C.sativum* against 186 bacterial isolates belonging to 10 different genera of gram positive bacterial population and two isolates *Candida albicans* from urine specimens have been observed by Saeed and Tariq (2007). *C.sativum* seeds used to treat hyperglycemia and hyperlipidemia, on endocrine functions and structures (Al–Suhaimi, 2009). It has been
documented as a traditional treatment for diabetes as well as a folk medicine for the relief of anxiety and insomnia in Iran (Chithra and Leelamma, 1997). Medicinally, coriander is used for minor digestive problems and externally for hemorrhoids and painful joints where it is rich in vitamins, decanal, nonanal, linalool and many fine substances. Acetone extract of coriander has shown the highest inhibitory activity against \textit{E.coli} followed by the highest activity against \textit{Pseudomonas} spp. with methanol extracts (Silva \textit{et al.}, 2011).

\subsection*{2.10.3. \textit{Curcuma} longa

Turmeric (\textit{C.longa}) is a rhizomatous herbaceous perennial plant of the ginger family \textit{Zingiberaceae} and grows to a height of three to five feet. It has been cultivated extensively in Asia, India, China and other countries with a tropical climate. It has oblong, pointed leaves and funnel shaped yellow flowers. The rhizome, shown in Figure 2.4, is the portion of the plant used is medicinally after completing a process of including boiled, cleaned, dried and yielding a yellow powder. Turmeric has been used extensively in foods for its flavour and colour as well as having a long tradition of use in the Chinese and Ayurvedic systems of medicine. It could be the major medicine for an anti-inflammatory as well as the treatment of flatulence, jaundice, menstrual difficulties, haematuria, haemorrhage and colic (Leung, 1980). All these salient futures of \textit{C.longa} have been inviting the special attention of researchers on turmeric’s pharmacological properties.

\subsubsection*{2.10.3.1. Taxonomical classification of \textit{C.} longa

Kingdom - Plantae
Subkingdom - Viridaeplantae
Infra kingdom - Streptophyta
Division - Tracheophyta
Subdivision - Spermatophytina
Infra division - Angiospermae
Class - Magnoliopsida
Super order - Lilianae
Order - Zingiberales
Family - Zingiberaceae
Genus - Curcuma \textit{L}
Species - Curcuma longa \textit{L}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{Figure_2.4.png}
\caption{Rhizome of \textit{Curcuma} longa}
\end{figure}

\subsection*{2.10.3.2. Pharmacological activities of \textit{C.} longa

In India, turmeric has been widely used for medicinal purposes for centuries. Current science has evidenced that curcumin would have an anti-inflammatory and anticancer
activities there by making the renewed scientific interest in its potential to prevent and treat the disease. It is a common spice, known mostly for its use in Indian dishes as a common ingredient in curries and other ethnic meals. This extraordinary herb would pave a way to spotlight in the west as it provides a wide range of medicinal benefits. Due to presence of obtainable quantity of vitamins C, E and Beta-Carotene in Curcumin, it acts as a powerful antioxidant for cancer prevention, liver protection and premature aging. Curcumin has the varieties of antioxidant, anti-inflammatory, antiviral and antifungal actions. Studies have revealed that Curcumin is not to be a toxic one to humans. Curcumin exerts anti-inflammatory activity by inhibition of a number of different molecules that play an important role in inflammation and also too effective in reducing post-surgical inflammation. Turmeric helps to prevent atherosclerosis by reducing the formation of blood clumps. Curcumin inhibits the growth of Helicobacter pylori, which causes gastric ulcers. Turmeric extract and the essential oil of C. longa inhibit the growth of a variety of bacteria, parasites, and pathogenic fungi. Curcumin has also been found to have moderate activity against Plasmodium falciparum and Leishmania major organisms as reported by Akram et al. (2010).

2.10.4. Emblica officinalis

The plant genus Phyllanthus (Euphorbiaceae) is widely distributed in most tropical and sub tropical countries of China, India, Indonesia, and on the Malay Peninsula. It is a very large genus consisting of approximately 550 to 750 species and is subdivided into 10 or 11 subgenera such as Botryanthus, Cicca, Conani, Emblica, Ericocus, Gomphidium, Isocladus, Kirganelia, Phyllanthodendron, Phyllanthus, and Xylophylla (Calixto et al., 1998). Phyllanthus emblica L. is a tree of small or moderate size with a greenish-grey bark and greenish-yellow flowers, formed in auxiliary clusters. The feathery leaves are linear-oblong, with a rounded base and obtuse or acute apex. The tender fruits are green, fleshy, globose and shining, and changed to light yellow or brick-red when matured. In Tamil, the tree is known as Nelli, the fruit is Nellikai (Figure 2.5) and in Bangladesh Amlaki, Amla in Hindi, and Yeowkan in Chinese. The fruits are also known as Amalakam and Sriphalam in Sanskrit, Emblic myrobalan and Indian gooseberry in English, and Phylontha emblic in French.

2.10.4.1. Taxonomical classification of E. officinalis

<table>
<thead>
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<tr>
<td>Class</td>
<td>Magnoliopsida</td>
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</tbody>
</table>

Figure 2.5. Fruit of Emblica officinalis
2.10.4.2. Pharmacological activities of *E. officinalis*

*E. officinalis* is one of the most extensively studied plants due to its selective contents of tannins, alkaloids, and phenolic compounds. It has also been reported that fruits of *E. officinalis* contains higher amount of vitamin C and considerably higher concentrations of most minerals, protein and amino acids like glutamic acid, proline, aspartic acid, alanine, cystine and lysine. The levels of vitamin C are found to be more than those in oranges, tangerines, or lemons (Krishnaveni and Mirunalini, 2010). Fresh pericarp of *E. officinalis* contains higher amount of hydrolysable tannins like emblicanin A and B, punigluconin and pedunculagin. The fruit also contains gallic acid, ellagic acid, chebulinic acid, chebulagic acid, emblicanin A, emblicanin B, punigluconin, pedunculagin, citric acid, ellagotannin, trigallayl glucose, pectin, 1-O-galloyl-β-D-glucose, 3,6-di-O-galloyl-D-glucose, chebulagic acid, corilagin, 1,6-di-O-galloyl-β-D-glucose, 3 ethylgallic acid (3 ethoxy 4,5 dihydroxy benzoic acid), and isostrictinin. In spite of these major constituents, it also contains flavonoid such as quercetin, kaempferol 3 O-α-L (6" methyl) rhamnopyranoside and kaempferol 3 O-α-L (6" ethyl) rhamnopyranoside (Patel and Goyal, 2012). *E. officinalis* is also stated to have the properties like hepatoprotective, cardioprotective, diuretic, laxative, refrigerant, stomachic, restorative, alterative, antipyretic, and anti-inflammatory properties. Besides being a hair tonic, *E. officinalis* also prevents peptic ulcer dyspepsia, and digestive problems (Baliga and Dsouza, 2011; Varghese et al., 2013). Aqueous infusion and decoction of *E. officinalis* exhibits potent antibacterial activity against *E.coli*, *Klebsiella pneumoniae*, *K. ozaenae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. paratyphi A*, *S. paratyphi B* and *Serratia marcescens* (Saeed and Tariq, 2007). The extracts of *E. officinalis* could serve as antimalarial agents even in their crude form (Bhagavan et al., 2011). Pinmai et al. (2010) found in vivo antiplasmodial activity in the extracts of *E. officinalis*. It possesses antiviral mechanisms so as to be a candidate for HSV therapy (Xiang et al., 2011), anticancer therapy (Ngamkitidechakul et al., 2010) and therapy for bone diseases (Penolazzi et al., 2008).
2.10.5. *Terminalia chebula*

*Terminalia* tree is usually tall about 50 to 80 feet in height with round crown and spreading branches. The bark is dark brown with some longitudinal cracks. Leaves are ovate and elliptical, with two large glands at the top of the petiole. The flowers are monoecious, dull white to yellow, with a strong unpleasant odour, born in terminal spikes or short panicles. The flowers appear by the month between May and June where as the fruits appear from July to December. The fruit or drupe is about 1 to 2 inches in size and five lines or five ribs on the outer skin. The green color unripened fruit become yellowish green when ripened. Fruits (Figure 2.6) have been collected from January to April, as well as the fruit formation started from November to January (Govt. of India, 2001). *T. chebula* is usually found in the Sub Himalayan tracks from Ravi eastwards to West Bengal and Assam, ascending upto the altitude of 1500 m in the Himalayas. This tree is wild in forests of Northern India, central provinces and Bengal, common in Madras, Mysore and in the southern part of the Bombay presidency (Gupta et al., 2012).

The fruit is likely to be mild laxative, stomachic, tonic, and alterative, antispasmodic. It is useful in ophthalmia, hemorrhoids, dental caries, bleeding gums and ulcered oral cavity. Its paste with water is found to be anti-inflammatory, analgesic and having purifying and healing capacity for wounds. Its decoction is used as gargle in oral ulcers and sore throat. Its powder is a good astringent dentifrice in loose gums, bleeding and ulceration in gums. It is good to increase appetite, digestive aid, liver stimulant, stomachic, gastrointestinal prokinetic agent, and mild laxative. The powder of *T. chebula* fruits has been used in chronic diarrhea, nervous weakness, and nervous irritability. It promotes the receiving power of five senses. It is adjuvant in hemorrhages due to its astringent nature and good for chronic cough, chorizo, sore throat as well as asthma. It is also useful in renal calculi, dysurea and retention of urine and skin disorders with discharges like allergies, urticaria and other erythematous disorders (Asolkar et al., 1992).

2.10.5.1. Taxonomical classification of *T. chebula*

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta</td>
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<tr>
<td>Superdivision</td>
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<tr>
<td>Subclass</td>
<td>Rosidae</td>
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<tr>
<td>Order</td>
<td>Myrtales</td>
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Figure 2.6. Dried fruit of *Terminalia chebula*
Family - Combretaceae
Genus - Terminalia L.
Species - Terminalia chebula

2.10.5.2. Pharmacological activities of *T. chebula*

The fruits of *T. chebula* are rich in tannins (about 32% to 34%) and its content varies with geographical distribution (Kumar, 2006; Kumar *et al*., 2009). The tannins of *T. chebula* are of pyrogallol (hydrolysable) type. A group of researchers have found 14 components of hydrolysable tannins (gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl- \( \beta \)-D-glucose, 1,6-di-o-galloyl-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose, terchebulin) from *T. chebula* fruits. Other constituents include phenolics such as chebulinic acid, ellagic acid and anthraquinones. The leaves are found to contain polyphenols such as punicalin, punicalagin, terflavins B, C, and D (Bruneton, 1995; Juang *et al*., 2004; Han *et al*., 2006). The plant is found to contain phloroglucinol and pyrogallol, along with phenolic acids such as ferulic, p-coumaric, caffeic and vanillic acids. Some of the other minor constituents are polyphenols such as corilagin, galloyl glucose, punicalagin, terflavin A and maslinic acid (Williamson, 2002). Besides, fructose, amino acids, succinic acid, beta sitosterol, resin and purgative principle of anthraquinone are also present (Thakur *et al*., 2008; Tubtimdee and Shotipruk, 2011). Flavonol, glycosides, triterpenoids, coumarin conjugated with gallic acids called chebulin as well as other phenolic compounds have also been isolated (Yoganarasimhan, 2000; Rangsriwong *et al*., 2009; Muhammad *et al*., 2012). Twelve fatty acids have been isolated from *T. chebula*, of which palmitic acid, linoleic acid and oleic acid are found to be major constituents (Zhang *et al*., 1997). Triterpenoid glycosides such as chebulosides I and II, arjunin, arjunglucoside, 2α-hydroxyursolic acid and 2α-hydroxymicromiric acid have been reported by Mammen *et al.* (2012). Oil extracted from kernels has yielded palmitic, stearic, oleic, linoleic, behenic and arachidic acids (Khare, 2004).

*T. chebula* Retz. is one of the most versatile plant having a wide spectrum of pharmacological and medicinal activities. This versatile medicinal plant is the unique source of various types of compounds with diverse chemical structure. Though it has a number of pharmacological activities due to the presence of various types of bioactive compounds, very little work has been done on the plausible medicinal applications of this plant against the diseases particularly on multidrug resistant bacterial pathogens. Hence extensive investigation is needed to exploit their therapeutic ability to combat diseases including drug
resistant infections. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, a drug development programme should be undertaken to develop modern drugs with the compounds isolated from *T. chebula* effective against different types of diseases (Bag *et al.*, 2013). Kumar *et al.* (2013) revealed that methanolic extract of *Terminalia chebula* fruit has better efficacy and to be a source for natural antimicrobial agent.

The ethanolic extract of *T. chebula* fruit is also found to be effective against both gram positive and gram negative bacteria and proven itself as broad spectrum antimicrobial activity (Kannan *et al.*, 2009). Aqueous, alcoholic and ethyl acetate extracts of leaves of *T. chebula* have shown good effect on five pathogenic fungi as made an attempt by Shinde *et al.* (2011). The anti amoebic effect of a crude drug formulation of *T. chebula* has also investigated and reported positively by Sohni *et al.* (1995). In immuno modulation studies, humoral immunity by means of stimulating cell - mediated immune response without disturbing the counts of T - cell in the animals (Sohni and Bhatt, 1996). Acetone seed extract of *T. chebula* is also found to have well anti plasmodial activity in a study of Bhagavan *et al.* (2011). The fruit extracts of *T. chebula* showed inhibitory effects on human immunodeficiency virus-1 reverse transcriptase (Mekkaway *et al.*, 1995).

Literature has reported antibacterial (Singh *et al.*, 2012), antifungal (Shinde *et al.*, 2011), antioxidant (Chang *et al.*, 2011), mild laxative (Tamhane *et al.*, 1997), cytoprotective (Naik *et al.*, 2004), antiviral (Lee *et al.*, 2011), anti-diabetic (Kannan *et al.*, 2012), wound healing (Choudhary *et al.*, 2011), cardio tonic (Reddy *et al.*, 1990), anticancer (Saleem *et al.*, 2002), antimutagenic (Grover and Bala, 1992), immuno-suppressive (Lee *et al.*, 2005), anti-ulcer (Tamhane *et al.*, 1997), intoxication (Suchalatha *et al.*, 2004) and anaphylactic shock (Shin *et al.*, 2001) in the dried ripe fruits of *Terminalia chebula retzius*. In support of many researchers it was found out that the fruits of *T.chebula* possesses immunomodulatory, cytotoxic, radioprotective, anticaries, anticonvulsant, antihelmintic, hepatoprotective, retinoprotective, antiarthritic, antiaging, antiphyletic, antispasmodial, chemopreventive, anxiolytic, hypolipidemic, anticeptive, hypcholesterolemic, antispermatic, antidepressant, cutaneous wound healing and molluscicidal activities (Li *et al.*, 2011; Ashwini *et al.*, 2011; Gupta, 2012; Suryaprakash *et al.*, 2012; Chandrashekhar *et al.*, 2012). A regular intake of *T.chebula* fruits have been reported to prevent *Salmonellae* infection and reduce the risk of typhoid (Khan and Jain, 2009). The methanolic extract of *T chebula* has been found to be useful in controlling infectious diseases in diabetic patients (Kumar *et al.*, 2013) and the aqueous extract of fully ripped fruits of *T chebula* acts as a free radical
scavenger with potential antioxidant effects on erythrocytes of aged rats (Mahesh et al., 2009). It was demonstrated that the ethanolic extract of T.chebula fruit exhibits antidiabetic activity as it possesses insulin like action and ability to promote insulin release (Borgohain et al., 2012).

2.11. Role of High Performance Thin Layer Chromatography (HPTLC) in analyzing phytocomponents of plant materials.

Currently HPTLC has become a routine and popular analytical technique due to its advantages of low operating cost, high sample through put and need for minimum sample clean up. The major advantage is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis (Faiyazuddin et al., 2011). HPTLC chromatogram pattern comparison seems to be promising for finger printing the active compounds in plant extracts. The methods TLC and HPTLC have been applied for the identification, the assay and the testing for purity, stability, dissolution or content uniformity of raw materials (herbal, animal extracts, fermentation mixtures, drugs and excipients) and formulated products (pharmaceuticals, cosmetics and nutrients) (Biringanine et al., 2006). These flexible and cost effective techniques provide the advantage of the simultaneous processing of standards and samples together with versatile detection possibilities including great variety of post – chromatographic derivatization reagents. It has also been widely employed for the quantification of secondary metabolites in plants (Anandjiwala et al., 2007).

2.12. Impact of biofilms on the emergence of UTI

The National Institute of Health (NIH) reported that 65 to 80% of all microbial diseases are biofilm-based, causing many deaths and high health costs worldwide (Estrela et al., 2009). Biofilms have been defined in the literature as “microorganisms attached to a surface and covered with an exo polysaccharide of microbial origin” (Liedl, 2001).

Biofilm formation is a developmental process, which initially involves the adhesion of bacterial cells to a surface via various surface proteins and/or conditioning films. Once adhered to the surface, the bacteria undergo a variety of changes including the activation and down regulation of many genes and production of an exo polysaccharide that covers and protect the cells. The formation of biofilm is mediated by mechanical, biochemical and genetic factors. It enhances the virulence of the pathogen and their potential role in various infections like dental caries, cystic fibrosis, osteo necrosis, urinary tract infection and eye infection (Sritharan and Sritharan, 2004). Biofilms represent bacterial communities embedded in self-produced extracellular polymeric matrix that attached to a surface. The
microbial population comprising a biofilm can be made up of single or multiple bacterial species. The extracellular matrix is an intermediate environment for biofilm bacteria that stabilizes the three-dimensional biofilm structure and mediates bacterial adhesion (Flemming and Wingender, 2010).

Surface structures of bacterial cells, such as flagella, curli fibers, type I fimbriae, are involved in biofilm formation of *Escherichia coli* (Beloin, 2008). One major component of the exo polysaccharides (EPS) in *E. coli* is colanic acid (an exo polysaccharide) which forms a protective capsule surrounding the bacterial cell and also sustains the biofilm architecture (Danese *et al*., 2000). In most gram negative bacteria, lipopolysaccharide (LPS) is one of the major constituents of the outer leaflet of the outer membrane and it provides the structural integrity of the outer membrane. LPS consists of three distinct components such as the lipid A, which is the hydrophobic portion of the molecule anchored in the outer membrane, the O-antigen extending from the cell to the external environment, and the core oligosaccharide (core OS), which links the O-antigen to the lipid A. The main virulence determinant of LPS resides in the lipid A, which is recognized by the cell membrane protein TLR4/MD2 receptor as part of the innate immune response (Poltorak *et al*., 1998; Hoshino *et al*., 1999).

Although a great deal is well known about biofilm development, there are still unresolved questions concerning the initiation of biofilm formation on the surface of host tissues and the factors that determine the immune response towards the biofilm. Some recent studies have revealed that cyclic dinucleotides such as c-di-GMP are recognized by the pattern recognition receptors of the innate immune system, which activates type I interferon production by the host (McWhirter *et al*., 2009; Woodward *et al*., 2010; Blander and Sander, 2012). The innate immunity is the first line of host defense against infection that recognizes and provides a rapid response to pathogens. It has been suggested that bacterial cyclic dinucleotides act as a signal of the presence of an incipient biofilm that triggers the immune response (Valle *et al*., 2013).

The study conducted by Rijavec *et al*.(2008), demonstrated that 53% strains of UPEC were biofilm producers. There was a significant correlation between biofilm production and resistance to multiple drug antibiotics including nalidixic acid as reported by Suman *et al*., 2007; Solo *et al*., 2007; Pramodhini *et al*., 2012,. Research study demonstrated by Ghanwate (2012), *E.coli* is the most frequent isolate of UTI and have high propensity to form biofilm and poor antibiotic sensitivity to conventional antibiotics. Biofilm formation in *E.coli* may promote colonization and lead to increased rate of UTIs. Such infections may be difficult to treat as they exhibit multidrug resistance. There is a significant correlation of biofilm with
Multiple Drug Resistance. The proximity of cells within the biofilm can facilitate a plasmid exchange and hence enhance the spread of antimicrobial resistance (Watnick and Kotler, 2000). There was a considerable association between biofilm production and resistance to multiple antibiotics, the search for alternative products continues and natural phytochemicals isolated from plants used in traditional medicine are considered as suitable alternatives to synthetic chemicals (Palambo, 2009). India is sitting on a gold mine of well recorded and well practiced knowledge of traditional herbal medicine. Hence this is the right time to look into the profile of Indian medicinal plant in the international scenario of primary health care maintenance and to do researchers part for the exploration of the available rich biodiversity for nation’s wealth, the present research was undertaken.

When compared to gram negative organisms need less concentration of the compounds to kill them than gram positive organisms. It is believed that gram negative bacteria are more resistant to plant based antimicrobials than gram positive bacteria. This is because the gram negative bacteria have an effective permeability barrier, comprised of outer membrane, which restricts the penetration of antimicrobial compounds, which extrude plant extracts across this barrier. The single membrane of gram positive bacteria is considerably more accessible to permeation by plant extracts in a region where these bacteria have limited protection (Chanda and Kaneria, 2011).

During the last few years, efforts have been directed towards developing preventive strategies that can be used to disarm microorganisms without killing them. An innovative approach is the use of biocide–free anti biofilm agents with novel targets, unique modes of action and properties that are different from those of the currently used antimicrobials. In addition, one of the main advantages of plant derived compounds with potential pharmaceutical and medical applications is the lack of shared pathogens between plants and mammals like alkaloids, terpenoids, flavonoids and coumarins, peptides, glycosides and polyphenols. They may act in a variety os ways; antibiotics, allosteric regulators, catalysts, catalytic cofactors, regulatory activities of DNA, RNA and proteins, pigments, mutagens, anti mutagens, receptor agonists, antagonists, signal molecules, siderophores, detergents, metal complexing or transporting agents, pheromones, toxins and other interesting activities (Villa and Cappitelli, 2013).

One strategy for biofilm control receiving serious consideration on the basis of interference with bacterial cell-to-cell communication (quorum sensing). Because quorum sensing plays a vital role in infections caused by human, animal and plant pathogens, the
identification of mechanisms that disrupt this system is a hot topic in Microbiology (Defoirdt et al., 2013).

2.13. Influence of silver nanoparticles in UTI diagnosis

Green synthesis of noble metal nanoparticles is a greatly developing field in modern medicine with great potential for countries rich in biological diversity including India. Green nanomaterial synthesis techniques for medicinal applications are desired because of their biocompatibility and lack of toxic byproducts. Nanobiotechnology is a fast growing interdisciplinary area where nanotechnology extends the tools and technology platforms for the investigation and transformation of biological systems and in turn, biology contributes inspiring models and bio assembled components to nanotechnology (Ali et al., 2013). It has been recommended in the biomedical applications of nanoparticles (NPs) owing to their size and structural similarity to biological molecules (Murugan et al., 2013). The promising alternative of “green chemistry” exploits the intricate biological pathways and biological resources of living systems, including bacteria, fungi, algae, viruses, plants, and plant extracts, for the bioproduction of NPs (Faramarzi and Sadighi, 2013). It has been suggested that to achieve NP synthesis, techniques employing natural reagents such as biodegradable polymers, microorganisms, plant extracts, and sugars and vitamins as reductants and capping agents would be attractive (Kharissova et al., 2013). Though both plants and microbes are used for NP synthesis, plant extracts are preferable because of production advantages such as the lack of elaborate culture maintenance and the possibility of large-scale production (Panda et al., 2011).

Although the role of plant phytochemicals in nanostructure formation and causal chemical interactions is debatable, phytochemicals have been successfully used for the reduction of metals such as gold, silver, platinum, and zinc, and the subsequent synthesis of metallic NPs (Annamalai et al., 2013), which have the ability to fulfill the application-oriented demands of biomedicine and industry. These biological approaches employing plant extract-mediated NP synthesis are also considered creditable alternative tools since they ensure biocompatible, nontoxic, and in vivo applicable nanomaterials. Moreover, the demand is increasing for bio-NP production via highly stable, eco-friendly processes with no toxic chemicals, even during large-scale production (Prakash et al., 2013). Hence, opportunities are still available for developing large-scale green processes and employing natural products in the emerging areas of nanotechnology where NPs have important applications. Hence, a number of bio-inspired approaches employing plant extracts as bioreductants for the synthesis of AgNPs have been explored.
Silver nanoparticles probably have multiple mechanisms of antibacterial action, but due to the current dearth of knowledge on this subject, the exact basis for the activity of AgNps is still uncharacterized. Some studies have shown that AgNps release Ag+ ions significantly in the presence of water (Santoro et al., 2007; Asharani et al., 2008; Damm and Munstedt, 2008). Lok and coworkers calculated that approximately 12% of the silver is present in the ionic form, tightly associated with the oxidation layer (Lok et al., 2007). However, their experimental design makes it difficult to distinguish between the mechanisms of action of AgNps and dissolved Ag+ ions. Hence, it was suggested that nano-silver affects bacterial membrane permeability by attaching to the cell membrane surface and modifying the cell potential. Observation of large numbers of nanoparticles inside bacteria suggests that this is important to the antibacterial mechanism (Morones et al., 2005).

Currently, there is a constant need to develop eco-friendly processes for the synthesis of nanoparticles. The focus for synthesis of nanoparticles has shifted from chemical towards green chemistry (Vigneshwaran et al., 2007). Biosynthesis of nanoparticles using plant extracts is the favorite method of green, eco-friendly production of nanoparticles and exploited to a vast extent because the plants are widely distributed, easily available, safe to handle and with a range of metabolites (Kulkarani et al., 2012). As the antibacterial activity of medicinal plants becomes more recognized, an attempt is being made to establish the antibacterial activity of plant extracts used to synthesize Nps and the combined effect of the metal and the plant extract (Prakash et al., 2013).


Among metal nanoparticles with proven antimicrobial activity, those made of silver are particularly effective bactericidal agents (Seil and Webster, 2012). The antibacterial properties of silver have long been known and nanoparticles of this metal (AgNps) are believed to be less toxic than silver ions. In recent years, the application of AgNps in various fields has expanded considerably. AgNps have been successfully used in medical and pharmaceutical nano-engineering for the delivery of therapeutic agents, in chronic disease diagnostics and as part of sensors (Wong and Liu, 2010; Thiwawong et al., 2013). The comparison of the various nano-silver activities that have been studied is difficult because of differences in the chemistry and physical properties of the particles employed. Furthermore the bactericidal effect of AgNps is dependent on the size and shape of the particles (Panacek et al., 2006; Pal et al., 2007). The specific surface area of a dose of AgNps increases as the particle size decreases, allowing greater material interaction with the surrounding environment. In addition, triangular- shaped particles of silver display more bacterial killing
activity than rods or spherical particles (Pal et al., 2007). Other characteristics affecting the biological activity of nanoparticles are zeta potential and particle chemistry, with the former likely to play a significant role in the ability of particles to penetrate into the cell (Seil and Webster, 2012).

Bacterial biofilms are a serious medical problem. Due to the increasing ineffectiveness of conventional antibiotics, numerous alternative methods to combat bacterial biofilms are being considered. Silver nanoparticles have recently received an increased attention for their antimicrobial effects and possible clinical applications. Despite numerous studies conducted over the last decade there are still considerable gaps in our knowledge about the antimicrobial properties of AgNps. Furthermore, the precise basis of their antibacterial activity has yet to be defined. This is mainly due to the pleiotropic effects of nano-silver on bacterial cells, which suggests multiple mechanisms of action on several cellular targets. Nonetheless, the strong anti-biofilm effect of AgNPs is indisputable (Markowska et al., 2013).

2.15 Molecular docking of α - Haemolysin receptor with partial purified extracts of T.chebula

Bio-prospecting of Indian plants has provided evidence for modern researches for the use of plants against various diseases (Rekha et al., 2011). Among the above mentioned metabolites, gallic acid and ellagic acid were found to be potential antibacterial metabolites (Bag et al., 2012). Bacterial survival is controlled by targeting cell membrane receptors, or cytoplasmic targets, and or nuclear membrane proteins involved in cell division (Aishwarya et al., 2013).