3. Review of literature

Urinary Tract Infection

Urinary tract infection is one of the important causes of morbidity and mortality in Indian population, affecting all age groups across the life span. UTI is an inflammatory response of the urothelium due to bacterial invasion. UTI may involve only the lower urinary tract or both the upper and lower tract (Mignini et al., 2009). Urinary tract infections are caused by numerous etiological agents including *Escherichia coli*, *Staphlococcus saprophyticus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterococcus* spp. Of these, uropathogenic strains of the gram negative bacteria *E. coli* (UPEC) account for over 80% of UTIs (Ronald, 2002). A defining feature of many UPEC strains and other gram negative uropathogens is the presence of fimbriae. It is expressed on the surface of the bacteria. An uropathogenic strain of bacteria may contain numerous gene clusters for encoding fimbriae. There are two main fimbrial units that play prominent roles in the development of UTI, type 1 and P-fimbriae. Both fimbriae when exposed to renal and urinary bladder biopsies have been shown to elicit a stronger IL-6 response than nonfimbriated *E. coli* (Proft and Baker, 2009).

UTI is the most common bacterial infection in the industrialized world. Seven million patient visits per year accounts in the USA with total costs exceeding one billion dollars. Microbial infection affects almost all parts of the urinary tract. Urinary tract is the most frequent sites of bacterial infection in human (Walters et al., 2012). UTI is one of the common bacterial infections and are responsible for significant
morbidity and health care costs worldwide (Johnson and Russo, 2002). It is also a common hospital borne infection (Dame et al., 2005), accounting for as many as 35% of nosocomial infections and it is the second most common cause of bacteraemia in hospitalized patients (Shirishkumar et al., 2012).

Urinary tract infections have been estimated that symptomatic urinary tract infections (UTI) occurs in as many as Seven million visits to emergency units and 100,000 hospitalizations annually. Urinary tract infections are one of the most common types of bacterial infections in humans, occurring both in the community and health care settings which ranks the highest among the most common reasons that compel an individual to seek medical attention (Kolawale et al., 2009). Today it represents one of the most common diseases encountered in medical practices, affecting people of all ages, from the neonate to the geriatric age group (Kunin, 1994). UTI represents one of the most common diseases encountered in medical practice today with an estimated 150 million UTIs per annum worldwide (Karlowsky et al., 2002).

Urinary tract infections are the most frequent bacterial infection in women. They occur most frequently between the ages of 16 and 35 years, with 10% of women getting an infection yearly and 60% having an infection at some point in their lives (Gupta et al., 2007). Recurrences are common, with nearly half of people getting a second infection within a year. Urinary tract infections occur four times more frequently in females than males. Although UTIs occur in both men and women, clinical studies suggest that the overall prevalence of UTI is higher in women. Uncomplicated UTIs in healthy women have an incidence of 50/1000/year (De Backer et al., 2008). An estimated 50% of women experience at least one episode of UTI at
some point in their lifetime and between 20% and 40% of women have recurrent episodes (Rock et al., 2007). Approximately 20% of all UTIs occur in men (Griebling, 2007).

The commonly prevailing factors like promiscuity, peer group influence, pregnancy and low socio-economic status are common among young men and women living in urban centers play a pivotal role in causing UTI (Kolawale et al., 2009). It has been usually observed that UTI most commonly occurs in females and up to one-third of all women experience a UTI at some point during their lifetimes (Palac, 1986). According to Khalifa & Khedher (2009) the prevalence of UTI was recorded higher in females than in males. Females were predominant with UTI showing 73.37% of urine culture positivity whereas the male subjects showed only 26.92% of culture positivity. Among females, age groups of 21-30 years followed by 11-20 and 31-40 years and among male's age group 31-40 were predominant age groups in terms of incidence. Higher positivity was observed in the age group of 41-50 (10.8%) in males as compared with age group of 31-40 (8.20%) and overall female subjects predominated over males in terms of urine culture positivity especially the age group of 21-30 with 20.5% positivity (Rastogi et al., 2011). Several reports have also indicated that females are more prone to having UTIs than males (Kolawale et al., 2009), as the urethra is shorter in females than males thus being more readily and easily prone to micro-organisms (Inabo and Obanibi, 2006). UTIs occur more frequently in women than in men, and will occur in roughly half of women during their lifetime. UTI are most common bacterial infection in women (Colgan and Williams, 2011). Abu-Taha and Swileh (2011) reported that 81.6 % UTI incidence was reported in Female and 18.4% were in male.
Causative agents of UTI

The most common pathogen causing UTIs is *Escherichia coli* (*E. coli*). They are called Uropathogenic *E. coli* (UPEC). UPEC strains are the primary and leading cause of UTI (Hilbert *et al.*, 2012; Walters *et al.*, 2012). They are responsible for greater than 80% of uncomplicated cases in adults (Walters *et al.*, 2012). Lebanon report stated that *E. coli* was the frequent isolate throughout 10 years (2000-2009) (Daoud and Afif, 2011). Canadian report also reported higher incidence of *E. coli* in UTI (Pitout, 2012). Navidinia *et al.*, (2012) reported that EHEC is responsible for infant and childrens UTI.

UPEC are the leading cause of both acute and chronic UTI. They often secrete a labile pore forming toxin known as $\alpha$ haemolysin (Dhakal and Mulvey, 2012). Iranian report also exhibited that the UPEC are the principal cause of UTI (Bagherpour *et al.*, 2011). According to Bourjilat *et al.*, (2011) from Moracco indicated that ESBL producing *E. coli* are an increasingly significant cause of community acquired infection world wide. One of the Mexican report also indicated the increasingly prevalence of UPEC (Molina-Laape *et al.*, 2011). The table 3.1 briefly describes incidence nature of UPEC in different countries.
Table 3.1
Incidence of UTI from different countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Percentage of UPEC Incidence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>60.7</td>
<td>Muvunyi et al., 2011</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>Ismaili et al., 2011</td>
</tr>
<tr>
<td>Bloemfontein</td>
<td>75</td>
<td>Bosch et al., 2011</td>
</tr>
<tr>
<td>Canada</td>
<td>&gt;60</td>
<td>Hidalgo et al., 2011</td>
</tr>
<tr>
<td>China</td>
<td>40</td>
<td>Liu et al., 2011</td>
</tr>
<tr>
<td>Columbia</td>
<td>60.1</td>
<td>Castro-Orozco et al., 2010</td>
</tr>
<tr>
<td>Croatia</td>
<td>67.7</td>
<td>Ili et al., 2011</td>
</tr>
<tr>
<td>Denmark</td>
<td>80</td>
<td>Custovi &amp; Sosa, 2011</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>Ejrns, 2011</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>80</td>
<td>Kibret and Abera, 2011</td>
</tr>
<tr>
<td>France</td>
<td>80</td>
<td>Lavigne et al., 2011</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>Chassin et al., 2011</td>
</tr>
<tr>
<td>Germany</td>
<td>80</td>
<td>Wieser et al., 2012</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>Hrl, 2011</td>
</tr>
<tr>
<td>Korea</td>
<td>81.4</td>
<td>Yoon et al., 2011</td>
</tr>
<tr>
<td>Pakistan</td>
<td>51</td>
<td>Iqbal et al., 2010</td>
</tr>
<tr>
<td>Poland</td>
<td>75.6</td>
<td>Chlabicz et al., 2011</td>
</tr>
<tr>
<td>Singapore</td>
<td>74.5</td>
<td>Bahadin et al., 2011</td>
</tr>
<tr>
<td>Spain</td>
<td>&gt;60</td>
<td>Soto et al., 2011</td>
</tr>
<tr>
<td></td>
<td>&gt;45</td>
<td>Hidalgo et al., 2015</td>
</tr>
<tr>
<td>Sweden</td>
<td>72</td>
<td>Jonsson et al., 2011</td>
</tr>
<tr>
<td>Switzerland</td>
<td>80</td>
<td>Scharenberg et al., 2011</td>
</tr>
<tr>
<td>Sydney</td>
<td>45.5</td>
<td>Kudinha et al., 2012</td>
</tr>
<tr>
<td>Turkey</td>
<td>81.7</td>
<td>Ipek et al., 2011</td>
</tr>
<tr>
<td>Uganda</td>
<td>57.5</td>
<td>Mwaka et al., 2011</td>
</tr>
<tr>
<td>UK</td>
<td>75.7</td>
<td>Crosby &amp; Faithfull, 2011</td>
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<tr>
<td>USA</td>
<td>80</td>
<td>Reiss and Mobley, 2011</td>
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<td></td>
<td>90</td>
<td>Spurbeck et al., 2011</td>
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<td></td>
<td>&gt;80</td>
<td>Schwartz et al., 2011</td>
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<td></td>
<td>90</td>
<td>Tchesnokova et al., 2011</td>
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<tr>
<td></td>
<td>75</td>
<td>Al-Hasan et al., 2011</td>
</tr>
</tbody>
</table>

Daoud and Afif (2011) reported that *E. coli* was the most frequent isolate throughout the ten years of study (60.64% of the total isolates). It was followed by *Klebsiella pneumoniae* and *Proteus* sp., *Pseudomonas aeruginosa*, *Enterococcus* sp., and *Streptococcus agalactiae*. *E. coli* occurred more frequently in women (69.8%) than in men (61.4%). Bacteriuria was found in 159 of 163 urine samples (98%).
Enterococcus faecalis and Escherichia coli were the most common species, one or both being detected in 72% of the urine samples, while Proteus species were found in 10% and a single isolate of Providentia species was seen (Jonsson et al., 2011).

**Clinical manifestations**

The clinical manifestations of UTIs can vary significantly, especially in the extremes of age. UTIs in children can present with different symptoms. Symptoms in children younger than 2 years of age tend to be nonspecific, and can include fever, vomiting, and failure to thrive. In contrast, the elderly patient who has a UTI may be asymptomatic. When symptoms are present, they can include abdominal pain or mental status changes. However, the classic symptoms of acute uncomplicated cystitis include dysuria, change in urinary frequency, urinary urgency, hematuria, and suprapubic pain. Fever is usually absent in those with lower UTIs. In general, acute uncomplicated pyelonephritis classically presents with flank pain, abdominal pain, nausea, vomiting, fever, and costovertebral angle tenderness. Symptoms of cystitis may or may not be present in those with pyelonephritis. When present, these signs can occur 24-48 hours prior to appearance of symptoms of pyelonephritis. Some patients with acute pyelonephritis can present with sepsis.

**Pathogenesis**

Uropathogens have characteristics that enable them to be successful in causing infections of the urinary tract. Adhesins enable the attachment to host membranes. Capsular polyaccharides, hemolysins, cytotoxic necrotizing factor (CNF) protein, and aerobactins are other factors that enable uropathogens to invade the urinary tract. Most uropathogens originate in the rectal flora, colonize the periurethral area and urethra, and
ascend to the bladder. Increasing evidence suggests that alteration of the normal vaginal flora, especially loss of H\textsubscript{2}O\textsubscript{2}-producing lactobacilli, may predispose women to introital colonization with Uropathogenic *Escherichia coli* (UPEC), which is responsible for 85% of infections in ambulatory patients and 50% of nosocomial infections (Mulvey *et al.*, 2001). Although the vast majority of UPEC are cleared by host defenses within a few days, small clusters of intracellular bacteria have occasionally been observed to persist for months in an antibiotic-insensitive state (Schaeffer *et al.*, 1981). It has been shown in a murine model of UTI that are uropathogenic in nature.

Normal physiological changes in levels of GAGs may render bladders more vulnerable to invasion by bacteria. Even in healthy young women with presumably unperturbed epithelia, bladder GAG levels may vary during the normal menstrual cycle. Furthermore, certain UPEC strains may contain virulence factors that allow the bacteria to penetrate into the transitional cells and form QIRs. Establishment of QIRs throughout the underlying transitional epithelium may predispose an individual to an increased likelihood of recurrence and may account for some of the frequent same-strain recurrences that are seen clinically despite appropriate antibiotic therapy.

As many as, 50% of the women report to have had at least one UTI in their lifetime. UTI is normally an ascending infection (less frequently UTI can be an infection through the bloodstream) where the bacteria derive from the patient’s own faecal flora. The initial step of the pathogenesis is colonization of the distal urethra and vagina in women. From the urethra, the pathogens may gain entrance into the bladder. Here, when the natural defense mechanisms e.g., flushing of urine, secretion of IgA or uromucoid (specific protein, a urinary mucoprotein, insoluble, or glycoprotein of
Tamm-Horsfall; type 1 pili link on it, so *E. coli* aggregate and are eliminated by urinary flux), are overwhelmed by the virulent bacteria, bacterial adhesion and colonization may occur evolving into UTI. The colonization of the urinary tract provokes cellular responses e.g., activation of the epithelial cells, secretion of cytokines and neutrophile migration into the urothelium. Therefore, the ability of some pathogens to overcome these mechanisms and colonize the urinary tract is linked to the presence of virulence factors encoded by horizontally acquired genes not present in their non-pathogenic relatives. These factors include adhesins, cytotoxins, iron-uptake systems and extracellular polysaccharides such as lipopolysaccharide and capsules (Sobel and Kaye, 2005).

**Diagnosis**

The urinalysis is the most important initial study in the evaluation of a patient suspected of having a UTI by history. A negative urinalysis makes the diagnosis of UTI extremely unlikely. A specimen should be collected by the “clean-catch” method to minimize likelihood of contamination (Barker *et al.*, 1991) or by catheterization when the “clean-catch” method is impossible. A finding by microscopic examination using a high-power lens of bacteria of more than seven white cells/mm³ in unspun urine or more than two white cells per high-power field in spun urine is consistent with an UTI. The leukocyte esterase test has sensitivity for defining UTI of between 62 and 68 %, with a positive predictive value of only 46-55 % and a negative predictive value of 88-92 % (Pfaller and Koontz, 1985). A nitrite test has a sensitivity of 35-85 % and specificity of 92-100 % for the presence of bacteria (Pappas, 1991). The leukocyteesterase nitrite combination has a sensitivity of 79.2 %, a specificity of 81 % and a negative predictive value of 94.5 % for specimens with $\geq 10^5$ CFU/ml (Pfaller and
Koontz, 1985). A combination of findings (i.e., bacteriuria, pyuria and a positive nitrite test) is more highly predictive for UTI (Bailey, 1995).

Escherichia coli (62.75%) was the predominant isolate followed by *Klebsiella pneumoniae* (10.78%), *Staphylococcus aureus* (9.80%), Coagulase negative *Staphylococcus aureus* (CoNS) (5.88%), *Enterococcus* sp (3.92%), *Klebsiella oxytoca* (2.00%), *Pseudomonas aeruginosa* (2.00%), *Proteus mirabilis* (2.00%) and *Proteus vulgaris* (1.00%). Multidrug resistance was observed in 68.82% of the total bacterial isolates (Shakya et al., 2014).

The rate of urinary tract infection among women (11 of 215, 5.1%) was significantly higher than that observed among men (1 of 215, 0.5%) (Kawano et al., 2015). During the study period, there were 431,461 reports for *E. coli*, 23,786 for *K. pneumoniae* and 6,985 for *P. aeruginosa* from urine specimens. These represented 61%, 3% and 1%, respectively, of all organisms isolated from urine specimens (Ironmonger et al., 2015).

A total of 420 consecutive patients with 599 isolates were identified. Most patients were ≥65 years old and women (75.4%), and 114 patients (27.1%) had bacteremia. *Escherichia coli* (69%) was the most common organism. Cefazolin was effective against *E. coli*, *K. pneumoniae*, and *P. mirabilis* in greater than 80% of the cases (Chen et al., 2013).
Escherichia coli

*Escherichia coli* (*E. coli*) are special bacterium because of the diversity of *E. coli* pathotypes and different types of infection caused. *E. coli*, a Gram-negative bacterium of the family Enterobacteriaceae is a commensal bacterium of the intestinal flora in man and warm-blooded animals. It represents at least 80% of the aerobic flora. *E. coli* is a heterogeneous bacterial species with high genomic plasticity and many pathogenic variants (Hacker and Kaper, 2000). The different pathovars of *E. coli* are characterized by their host tropism or tissue. Some strains are responsible for intestinal infections causing severe diarrhoea. Other strains cause extraintestinal infections such as urinary tract infections, newborn meningitis, septicaemia, pneumonia but also systemic infections in poultry. These bacteria must be able of infecting the host, to resist the immune system and later to efficiently colonize these different niches (Ron, 2006).

Among extraintestinal pathogenic *Escherichia coli* (ExPEC) strains, uropathogenic *E. coli* (UPEC) are responsible for approximately 90% of uncomplicated urinary tract infections (UTI) and about 50% of the nosocomial UTIs (Rubin, 1990; Svanborg and Godaly, 1999).

Origin of *Escherichia coli*

The German pediatrician Theodor Escherich described a bacterial species isolated from stool of a healthy newborn as a Gram-negative rod of about 1.1-1.5μm x 2.0-6.0 μm. Theodor Escherich considered it as a typical “colonic bacterium” and designated it “Bacterium coli commune”. In 1919 “Bacterium coli commune” was re-
named into *Escherichia coli* (*E. coli*) in honor of the man who discovered it. This denomination became official in 1958 on recommendation of the subcommittee Enterobacteriaceae of the nomenclature committee of the International Association of Microbiology Societies (Bettelheim, 1986 and Escherich, 1989).

Escherich was initially convinced that “Bacterium coli commune” is a “harmless ommensal”. However, Escherich reported “on cystitis in children provoked by “Bacterium coli commune” as early as 1894 in a published lecture. He hypothesised that the intestinal bacteria could be considered as a source of urinary tract infections (bladder and kidney infections). This early hypothesis that *E. coli* bacteria which persist without symptoms in the intestine and for various reasons find their way into the urinary tract where they might cause inflammation, has now been confirmed by modern biochemical and molecular biological methods (Hacker et al., 1983). Escherich already implicitly put the potential pathogenicity of *E. coli* in observing the strong frequency of neonatal diarrhea and lethality in rabbits and guinea pigs infected by these bacteria.

Escherich himself observed the morphological variety of *E. coli* colonies. Even more numerous are the serological variants. The *E. coli* strains are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigen profiles, where the specific combination of these factors defines the serotype of an isolate. *E. coli* strains of a specific serotype can be associated with certain clinical manifestations. However, the surface antigens alone are not considered to confer pathogenicity themselves. Rather there are specific clonal lineages which have served as “hosts” for horizontally transferred virulence genes resulting in pathogenic clones (Zingler et al., 1992).
**E. coli Infections**

Pathogenic *E. coli* bacteria are classified into different “pathotypes” according to the disease type they cause. *E. coli* strains causing intestinal infections are distinguished from other strains that are responsible for extraintestinal infections. Pathogenic *E. coli* variants are characterized by the presence of various virulence factors, such as various toxins, particular fimbrial adhesins, invasins or secretion system. They can be present in the bacterial genome, encoded on genomic/pathogenicity islands, plasmids or phages.

**E. coli involved in intestinal infections**

Intestinal pathogenic *E. coli* are subdivided at present into six pathotypes which cause diarrhoea with different clinical manifestation (Kaper et al., 2004). The clinical symptoms and the virulence factors expressed by the strains, the adhesion factors and toxins, are used as criteria for their classification. The serotyping mainly employed in earlier years to identify and classify clinical isolates is being more and more replaced today by the molecular genetic detection of bacterial virulence and pathogenicity factor-encoding genes that are known, supported by evidence of specific pathogenic features.

**Enteropathogenic E. coli (EPEC)**

EPEC was the first pathotype of *E. coli* to be described. EPEC remains an important cause of potentially fatal infant diarrhoea in developing countries (Nataro and Kaper, 1998). A characteristic intestinal histopathology is associated with EPEC infections; known as ‘attaching and effacing’ (A/E), the bacteria intimately attach to
intestinal epithelial cells and cause striking cytoskeletal changes, including the accumulation of polymerized actin directly beneath the adherent bacteria. The microvilli of the intestine are effaced and pedestal-like structures on which the bacteria perch frequently rise up from the epithelial cell, a 35-kb pathogenicity island (PAI) (McDaniel et al., 1995). Homologues of LEE are also found in other human and animal pathogens. The model of EPEC pathogenesis is considerably more complex than simple binding to epithelial cells by a single adhesin and secretion of an enterotoxin that induces diarrhoea. EPEC initially adhere to epithelial cells by an adhesion.

**Enterotoxigenic E. coli (ETEC)**

ETEC cause watery diarrhoea, which can range from mild, self-limiting disease to severe purging disease. The organism is an important cause of childhood diarrhoea in the developing world and is the main cause of diarrhoea in travellers to developing countries (Nataro and Kaper, 1998). ETEC colonizes the surface of the small bowel mucosa and secrete enterotoxins, which induce intestinal secretion. ETEC enterotoxins belong to one of two groups: the heat-labile enterotoxins (LTs) and the heat-stable enterotoxins (STs). ETEC strains might express only an LT, only an ST, or both LTs and STs. LTs are a class of enterotoxins that are closely related in structure and function to cholera enterotoxin (CT), which is expressed by *Vibrio cholerae* (Spangler, 1992). STs are small, single-peptide toxins that include two unrelated classes — STa and STb — which differ in both structure and mechanism of action. Only toxins of the STa class have been associated with human disease (Nataro and Kaper, 1998). The STb toxin is associated with animal disease and is a 48-amino acid peptide containing two disulphide bonds. STb can elevate cytosolic Ca\(^{2+}\) concentrations, stimulate the release
of prostaglandin E2 and stimulate the release of serotonin, all of which are mechanisms that could lead to increased ion secretion. ETEC is mainly a pathogen of developing countries, and it is well known that these countries typically have a low rate of colon cancer. Pitari et al., (2003) have reported that STa suppresses colon cancer cell proliferation through a guanylyl cyclase C mediated signalling cascade. Accordingly, the high prevalence of ETEC in developing countries might have a protective effect against this important disease, and indicates that infectious diseases might exist in a complex evolutionary balance with their human populations.

**Enterohaemorrhagic E. coli (EHEC)**

EHEC causes bloody diarrhoea (haemorrhagic colitis), non-bloody diarrhea and haemolytic uremic syndrome (HUS). The bovine intestinal tract is the principal reservoir of EHEC and initial outbreaks were associated with consumption of undercooked hamburgers. Subsequently, a wide variety of food items have been associated with disease, including sausages, unpasteurized milk, lettuce, cantaloupe melon, apple juice and radish sprouts — the latter were responsible for an outbreak of 8,000 cases in Japan. Facilitated by the extremely low infectious dose required for infection (estimated to be <100 cells), EHEC has also caused numerous outbreaks associated with recreational and municipal drinking water, person-to-person transmission and petting zoo and farm visitations. A recent report indicates potential airborne transmission after exposure to a contaminated building (Varma et al., 2003). EHEC strains of the O157:H7 serotype are the most important EHEC pathogens in North America, the United Kingdom and Japan, but several other serotypes, particularly those of the O26 and O111 serogroups, can also cause disease and are more prominent than O157:H7 in many countries. The key virulence factor for EHEC is Stx,
which is also known as verocytotoxin (VT). The Stx family contains two subgroups — Stx1 and Stx2 — that share approximately 55% amino acid homology. Stx is produced in the colon and is transported by the bloodstream to the kidney, where it damages renal endothelial cells and occludes the microvasculature through a combination of direct toxicity and induction of local cytokine and chemokine production, resulting in renal inflammation (Andreoli et al., 2002). This damage can lead to HUS, which is characterized by haemolytic anaemia, thrombocytopenia and potentially fatal acute renal failure. Stx also mediates local damage in the colon, which results in bloody diarrhoea, haemorrhagic colitis, necrosis and intestinal perforation. In addition to Stx, most EHEC strains also contain the LEE pathogenicity island that encodes a type III secretion system and effector proteins that are homologous to those that are produced by EPEC. Animal models have shown the importance of the intimin adhesin in intestinal colonization, and HUS patients develop a strong antibody response to intimin and other LEE encoded proteins. EHEC O157:H7 is believed to have evolved from LEE-containing O55 EPEC strains that acquired a bacteriophage encoding Stx (Reid et al., 2000). It is also responsible for UTI in infants and children (Navidinia et al., 2012).

**Enteroaggregative E. coli (EAEC)**

EAEC are increasingly recognized as a cause of often persistent diarrhea in children and adults in both developing and developed countries, and have been identified as the cause of several outbreaks worldwide. At present, EAEC are defined as *E. coli* that do not secrete LT or ST and that adhere to HEp-2 cells in a pattern known as autoaggregative, in which bacteria adhere to each other in a ‘stacked-brick’ configuration (Nataro and Kaper, 1998). Nevertheless, at least a subset of EAEC has been proven as human pathogens. The basic strategy of EAEC infection seems to
comprise colonization of the intestinal mucosa, probably predominantly that of the colon, followed by secretion of enterotoxins and cytotoxins (Nataro et al., 1998). Studies on human intestinal explants indicate that EAEC induces mild, but significant, mucosal damage (Hicks et al., 1996). Mild inflammatory changes are observed in animal models (Vial et al., 1988) and evidence indicates that at least some EAEC strains might be capable of limited invasion of the mucosal surface (Abe et al., 2001). The most dramatic histopathological finding in infected animal models is the presence of a thick layer of autoaggregating bacteria adhering loosely to the mucosal surface. EAEC prototype strains adhere to HEp-2 cells and intestinal mucosa by virtue of fimbrial structures known as aggregative adherence fimbriae (AAF) (Czeczulin et al., 1997; Nataro et al., 1992; Nataro et al., 1994), which are related to the Dr family of adhesins. Several toxins have been described for EAEC. Two such toxins are encoded by the same chromosomal locus on opposite strands. The larger gene encodes an autotransporter protease with mucinase activity called Pic; the opposite strand encodes the oligomeric enterotoxin that is known as Shigella enterotoxin 1, owing to its presence in most strains of Shigella flexneri 2a (Noriega et al., 1995). Although no single virulence factor has been irrefutably associated with EAEC virulence, epidemiological studies implicate a ‘package’ of plasmid-borne and chromosomal virulence factors, similar to the virulence factors of other enteric pathogens. Several EAEC virulence factors are regulated by a single transcriptional activator called AggR, which is a member of the AraC family of transcriptional activators (Nataro et al., 1994). One consistent observation from studies involving EAEC epidemiology is the association of the AggR regulon with diarrhoeal disease. Jiang et al., (2002) have recently shown that the presence of genes associated with the AggR regulon is predictive of significantly increased concentrations of faecal IL-8 and IL-1 in patients.
with diarrhoea caused by EAEC. It has been suggested that the term ‘typical EAEC’ should be reserved for strains carrying AggR and at least a subset of AggR regulated genes, and that the term ‘atypical EAEC’ should be used for strains lacking the AggR regulon.

**Enteroinvasive E. coli (EIEC)**

EIEC are biochemically, genetically and pathogenically closely related to *Shigella* sp. Numerous studies have shown that *Shigella* sp. and *E. coli* are taxonomically indistinguishable at the species level (Pupo *et al.*, 2000), but, owing to the clinical significance of *Shigella*, a nomenclature distinction is still maintained. The four *Shigella* species that are responsible for human disease, *S. dysenteriae*, *S. flexneri*, *Shigella sonnei* and *Shigella boydii*, cause varying degrees of dysentery, which is characterized by fever, abdominal cramps and diarrhoea containing blood and mucus. EIEC might cause an invasive inflammatory colitis, and occasionally dysentery, but in most cases EIEC elicits watery diarrhoea that is indistinguishable from that due to infection by other *E. coli* pathogens (Nataro and Kaper, 1998). EIEC are distinguished from *Shigella* sp. by a few minor biochemical tests, but these pathotypes share essential virulence factors. EIEC infection is thought to represent an inflammatory colitis, although many patients seem to manifest secretory, small bowel syndrome. The early phase of EIEC/*Shigella* pathogenesis comprises epithelial cell penetration, followed by lysis of the endocytic vacuole, intracellular multiplication, directional movement through the cytoplasm and extension into adjacent epithelial cells (Sansonetti and Parsot, 2000). Movement within the cytoplasm is mediated by nucleation of cellular actin into a ‘tail’ that extends from one pole of the bacterium. In addition to invasion
into and dissemination within epithelial cells, *Shigella* (and presumably EIEC) also induces apoptosis in infected macrophages (Zychlinsky *et al*., 1992).

Genes that contribute to this complex pathogenicity are present on a large virulence plasmid that is found in EIEC and all *Shigella* species. The sequence of the 213-kb virulence plasmid of *S. flexneri* (pWR100) indicates that this plasmid is a mosaic that includes genetic elements that were initially carried by four plasmids (Buchrieser *et al*., 2000). One-third of the plasmid is composed of insertion sequence (IS) elements, which are undoubtedly important in the evolution of the virulence plasmid. This plasmid encodes a type III secretion system and a 120-kDa outer-membrane protein called IcsA, which nucleates actin by the binding of N-WASP (Egile *et al*., 1999; Goldberg and Theriot, 1995). The growth of actin micofilaments at only one bacterial pole induces movement of the organism through the epithelial cell cytoplasm. This movement culminates in the formation of cellular protrusions that are engulfed by neighbouring cells, after which the process is repeated. Although EIEC are invasive, dissemination of the organism past the submucosa is rare. Although the extensively characterized type III secretion system is essential for the invasiveness characteristic of EIEC and *Shigella* sp, additional virulence factors have been described, including the plasmid-encoded serine protease SepA, the chromosomally encoded aerobactin iron-acquisition system and other secreted proteases that are encoded by genes present on pathogenicity islands.

**Diffusely adherent *E. coli* (DAEC)**

DAEC are defined by the presence of a characteristic, diffuse pattern of adherence to HEp-2 cell monolayers. DAEC have been implicated as a cause of
diarrhoea in several studies, particularly in children >12 months of age (Nataro and Kaper, 1998). DAEC strains induce a cytopathic effect that is characterized by the development of long cellular extensions, which wrap around the adherent bacteria. This characteristic effect requires binding and clustering of the DAF receptor by Dr Fimbriae (Bernet-Camard et al., 1996). All members of the Dr Family (including UPEC as well as the DAEC strain C1845) elicit this effect (Bilge et al., 1989). Binding of Dr Adhesins is accompanied by the activation of signal transduction cascades, including activation of PI-3 kinase (Peiffer et al., 1998). Peiffer et al., (2001) have reported that infection of an intestinal cell line by strains of DAEC impairs the activities and reduces the abundance of brush border-associated sucrase isomaltase and dipeptidylpeptidase IV. This effect is independent of the DAF-associated pathway described above, and therefore provides a feasible mechanism for DAEC-induced enteric disease and also indicates the presence of virulence factors in DAEC other than Dr Adhesins. Tieng et al., (2002) have proposed that DAEC might induce expression of MICA by intestinal epithelial cells, indicating that DAEC infection could be proinflammatory; this effect could potentially be important in the induction of inflammatory bowel diseases.

**Extraintestinal pathogenic Escherichia coli**

Extraintestinal infections involving *E. coli* include urinary tract infections, newborn meningitis as well as human and animal septicemia (Wieser et al., 2012). ExPEC are very often found as intestinal commensal flora and are not the cause of gastroenteritis in humans. The acquisition of ExPEC by the host does not often cause an infection, they will have to colonize first, from the intestine or external middle,
tissues and organs normally sterilized (urinary tract, peritoneal cavity, lungs) (Johnson and Russo, 2002). ExPEC strains express different types of virulence factors which allow them to colonize the surface of the mucous membranes of the host, to escape host defense mechanisms, to multiply under conditions of limited essential elements (nutrients) such as iron. Other virulence factors will enable ExPEC to invade the host tissue and to induce an inflammatory response (Johnson, 2003). These virulence factors of ExPEC include various adhesins, surface polysaccharides (capsule, LPS), toxins, siderophores, proteases, invasins and proteins enabling them to resist the effects of complement. It is impossible today to characterize with precision different pathotypes among ExPEC solely on the basis of their virulence factors. Nevertheless, ExPEC strains were isolated from various extraintestinal infections. Thus *E. coli* is responsible for urinary tract infections (UPEC, Uropathogenic *E. coli*).

Extraintestinal pathogenic *Escherichia coli* (ExPEC) are an important cause of urinary tract infections, neonatal meningitis and septicaemia in humans. Animals are recognized as a reservoir for human intestinal pathogenic *E. coli*, but whether animals are a source for human ExPEC is still a matter of debate.

**The UPEC pathotype**

UPEC strains are the most frequent pathogens responsible for 85% and 50% of community and hospital acquired UTIs, respectively. UPEC strains have special virulence factors, including type 1 fimbriae, which can result in worsening of UTIs (Hojati *et al.*, 2015).
It is a primary cause of UTI (Hilbert et al., 2012). The urinary tract is the most common site of bacterial infection in industrialized countries (Warren, 1996), and urinary tract infection (UTI) is also the leading nosocomial disease. UTI can be caused by several microbial pathogens. The most common causative agent of urinary tract infections, however, are uropathogenic *E. coli* (UPEC), which cause uncomplicated UTI in about 80% of all cases (Walters et al., 2012; Hooton and Stamm, 1997; Svanborg and Godaly, 1997). The urinary tract represents a usually sterile compartment, which is protected from bacterial infections by various mechanisms such as urine flow and immune responses. Furthermore, the urinary tract is a hostile environment in terms of supporting bacterial growth. The chemical composition, osmolarity and pH of urine determine the rate of bacterial growth and the maximum population that can be supported, and can be very variable, depending on the diet. Normal urine constituents include amino acids and glucose, which are usually present at sufficient concentrations to support rapid bacterial growth. However, other components of urine, such as urea and organic acids, may inhibit growth, mainly by affecting pH and osmolarity (Asscher et al., 1966). Therefore, the ability of some pathogens to overcome these mechanisms and colonize the urinary tract is linked to the presence of virulence factors encoded by horizontally acquired genes not present in their non-pathogenic relatives. These factors include adhesins, cytotoxins, iron-uptake systems and extracellular polysaccharides such as lipopolysaccharide and capsules.

The subset of *E. coli* that causes uncomplicated cystitis and acute pyelonephritis is distinct from the commensal *E. coli* strains that comprise most of the *E. coli* colonizing the lower colon of humans. *E. coli* from a small number of O serogroups (six O groups cause 75% of UTIs) have phenotypes that are epidemiologically associated with cystitis and acute pyelonephritis in the normal urinary tract, which include expression of P
fimbriae, α-haemolysin, aerobactin, serum resistance and encapsulation. Clonal groups and epidemic strains that are associated with UTIs have been identified (Nowicki et al., 1989; Phillips et al., 1988).

Strains of uropathogenic *E. coli* are responsible for approximately 90% of community-acquired, uncomplicated cystitis, and fimbriae represent the adhesive factors enabling *E. coli* to be anchored to uroepithelial cells in the first step of the infectious process (Tempera et al., 2010). *E. coli* infection is occurring in an ascending manner with bacterium travelling from the bladder to the kidneys and potentially the blood stream (Walters et al., 2012).

**Pathogenesis of urinary tract infection caused by uropathogenic *E. coli*.**

UPEC causes both acute and chronic UTI. It often secrete heat labile pore forming toxin known as α haemolysin (*hly A*). Hly A induces proteolysis of host protein. It not only modulates epithelial cell function, but also disables macrophages and suppresses inflammatory responses (Dhakal and Mulvey, 2012).

**Virulence factors of ExPEC**

Different potential virulence factors were identified in ExPEC, allowing them to adhere, to penetrate the epithelial cells, to resist to the immune system and to multiply. Virulence factors are responsible for the pathogenic potential of *E. coli* strains (Lane et al., 2007). Based on the availability of virulence factors, *E. coli* cells attach selectively to the mucosa uro-epithelium, promoting colonization and persisting in the urinary tract, inducing, then, a local inflammatory response and sometimes to promote tissue lesions (Mulvey, 2002).
P fimbriae, the principal mannose-resistant adherence organelles of extraintestinal pathogenic Escherichia coli, are known to contribute to pathogenesis by promoting bacterial colonization of host tissues and by stimulating an injurious host inflammatory response. *E. coli* strains were examined for *pap* genotype and specific primers were utilized to detect genes associated with outer membrane protein, minor structural subunit and adhesion but none of our isolates had *pap* gene (Kuehn *et al.*, 1994).

VF found in association with pyelonephritis, meningitis and sepsis is S fimbriae (Mulvey, 2002). S fimbrial adhesin recognizes surface sialic acid content, on receptors expressed by kidney epithelial and vascular endothelial cells, mediating bacterial adherence. Recently, it was identified sialic acid residues in the UP3, one of the four integral membrane uroplakin proteins, expressed on the bladder luminal surface, suggesting that S fimbriae may also have a role in cystitis (Malagolini *et al.*, 2000).

The majority of the UPEC strains present group II capsules (K1, K5) determined by *kps* operon. Capsule is common in UPEC and is better known for contributing with pyelonephritis than other urinary tract infections (Jann and Jann, 1997).

Kurazono *et al.*, (2000) reported a putative pathogenicity island (PAI), which was more frequently found in UTI collections than in fecal *E. coli*. This PAI contains *usp* that encodes a 346-amino acid protein, which was designated as uropathogenic specific protein (USP). Parret and De Mot (2002) hypothesized that the
uropathogenic specific protein may represent a novel type of *E. coli* bacteriocin, acting against competing *E. coli* strains that occupy the same niche, thereby enhancing their infectivity in the urinary tract environment. Recently, it was demonstrated that usp significantly enhanced the infectivity of *E. coli* in the mouse UTI model (Yamamoto, 2007).

**Fimbrial adhesins**

The fimbriae possess fibre-like structures and are visible on the bacteria surface by electronic microscopy. Fimbriae exhibit a composite structure, consisting of a rod shaped shaft of 6-7 nm in diameter comprising over a thousand major subunits and minor subunits. In ExPEC, four important types of fimbriae are distinguished, i.e. type 1 fimbriae, P fimbriae, S/F1C fimbriae (and AC/I fimbriae). P-, S- and F1C-fimbriae are more exclusively associated with extraintestinal *E. coli* isolates and the tip of these adhesins recognize carbohydrate moieties: Gala (1-4) Gal, a-sialyl-2, 3-b-galactose and GalNAcb (1-4) Galb, respectively. These fimbriae are factors contributing to the virulence potential of such strains, but they are not necessarily sufficient to cause disease (Mobley *et al.*, 1994).

**Type 1-fimbriae**

The type 1-fimbriae are extracellular structures encoded by a group of nine genes localized on the core chromosome (*fimA* to *fim I*) where seven genes are organized in an operon (*fimA, fimC, fimD, fimF-I*) whose expression is phase variable (McClain *et al.*, 1993). The *fimA* gene encodes the major component of the structure, the fibrillin. Other genes of the operon encode minor proteins, including *FimH*
encoding the adhesin. The role of the major subunits is yet unclear, although they have been proposed to be important for adherence to mammalian extracellular matrix proteins (Korhonen, 2000).

The adhesin and some other minor subunits are responsible for the specific binding to carbohydrate moieties on the surface of eukaryotic cells, therefore contributing to specific adherence. The syntheses, export, correct folding and ordered assembly during the fimbrial biogenesis occurs in a coordinated manner (Smyth et al., 1996). These fimbriae are characterized by their ability to adhere to and agglutinate erythrocytes of mammals and birds. This adhesion is inhibited by the addition of D-mannose which blocks the adhesion FimH. Accordingly, these fimbriae are also called mannose-sensitive hemagglutinating (MSHA) fimbriae (Vidotto et al., 1990). Type 1-fimbriae are present in both ExPEC and nonpathogenic strains. However, these fimbriae are more represented among pathogenic than among non-pathogenic strains and have been for a long time considered as potential virulence factors for ExPEC (McPeake et al., 2005).

The FimH gene was found in 130 isolates (92.8%) of the UPEC strains. Of 130 isolates positive for the FimH gene, 62 (47.7%) and 68 (52.3%) belonged to hospitalized patients and outpatients, respectively (Hojati et al., 2015). During UTI, between 4 and 24 hours after infection, the new environment in the bladder selects for the expression of type 1-fimbriae (Gunther et al., 2001), which play an important role early in the development of an UTI (Connell et al., 1996). Type 1-fimbriated E. coli attach to mannose moieties of the uroplakin receptors that coat transitional epithelial cells (Mulvey et al., 1998). Attachment triggers apoptosis and exfoliation; for at least
one strain, invasion of the bladder epithelium is accompanied with formation of pod-like bulges on the bladder surface that contain bacteria encased in a polysaccharide-rich matrix surrounded by a shell of uroplakin (Anderson et al., 2003). In strains that cause cystitis, type 1-fimbriae are continuously expressed and the infection is confined to the bladder (Connell et al., 1996).

Among APEC expressing type 1-fimbriae, the in vivo expression of these adhesins was highlighted in the trachea, the air sacs and lungs, but not in the blood or deep organs. This result suggested a potential role in the early stages of infection (Pourbakhsh et al., 1997).

P-fimbriae

Many ExPEC strains express P-fimbriae which are one of the most extensively studied adhesin, and also the first virulence-associated factor identified for UPEC. They were first described in E. coli isolated from urinary tract infections (pyelonephritis) in humans (Kallenius et al., 1981). P-fimbriae are heteropolymers encoded by a chromosomal locus of 11 genes (papA to papK) (Kariyawasam et al., 2006). The pap locus codes for the major protein papA and the adhesin papG that exists in three variants (Stromberg et al., 1991). These variants recognize different glycolipid isoreceptors and are recognizable by their ability to agglutinate different types of erythrocytes. As this haemagglutination is not inhibited in the presence of D-mannose, these fimbriae are also designated “mannose-resistant hemagglutination (MRHA) fimbriae” (Vaisanen et al., 1981).
P-fimbriae, encoded by the pap (pyelonephritis-associated pili) genes, are significantly prevalent among strains of UPEC that cause pyelonephritis and are characterized by their adherence to Galactose moieties present in the globoseries of membrane glycolipids on human ythrocytes of the P blood group and on uroepithelial cells (Lederberg, 1951). The PapG variant and the chromosomal location of pap alleles typically differ among UPEC strains. The pap gene clusters reside within pathogenicity islands. Since the discovery of P-fimbriae, it has been hypothesized that these adhesions contribute to the pathogenesis of UPEC within the mammalian urinary tract. More recent studies have uncovered a molecular crosstalk between the Toll-like receptor 4 that binds bacterial lipopolysaccharide and P-fimbrial-mediated attachment, which is lipopolysaccharide-independent. Activation of the Toll-like receptor 4 by P-fimbrial attachment subsequently leads to the production of pro-inflammatory cytokines and chemokines (interleukin-6 and CXCL8, respectively) and recruitment of neutrophils (Bergsten et al., 2005). Since P-fimbriae are implicated in triggering inflammation, it can be deduced that they may also contribute to the pathology and symptoms of acute pyelonephritis. It appears that there is a subtle role for P-fimbriae in mediating adherence to uroepithelial cells in vivo and establishing a robust inflammatory response during renal colonization, which in turn contributes to kidney damage during acute pyelonephritis.

**Iron acquisition systems**

Iron is necessary for growth of most microorganisms. The concentration of free iron available in body fluids animals and humans is much lower (about 10-18 mol/L) and does not cover the needs of the bacterium (Chipperfield and Ratledge, 2000). To remedy this lack, bacteria have developed two strategies to dispose of iron present in
eukaryotic cells. On the one hand, bacteria express receptors which can bind complexed iron as present in the host organism in proteins such as transferrin, lactoferrin but also in hemoglobin. Then, the bacteria can take this iron up and use it for growth. The second strategy is the synthesis of siderophors with high affinity for iron, allowing them to capture iron ions by competing with physiological chelators (Ratledge and Dover, 2000). The genes coding for the biosynthesis of such iron-uptake systems in E. coli may be located on plasmids or on the chromosome. The gene clusters encoding the enzymes for enterobactin and the ferric dicitrate transport system have a commonly conserved localization in the E. coli core genome. Another mechanism for iron acquisition in pathogenic E. coli is the direct utilization of host iron compounds, particularly heme or hemoglobin (Law and Kelly, 1995).

In UPEC, the aerobactin iron uptake system has been shown to contribute to serum resistance as well as to bacterial survival and growth in the host (Carbonetti et al., 1986). IroN, a novel catecholate siderophore receptor, has been shown to be more prevalent in E. coli isolates from UTI or bacteremia specimens than in fecal E. coli isolates (Russo et al., 1999).

**Phosphate transporter system (Pst)**

The Pst system is a phosphate ATP-dependent transporter, a family member of the ABC-transporters. The corresponding proteins are encoded by the operon pstSCAB-phoU which is located on the chromosome and belongs to the Pho regulon (Rao and Torriani, 1990). This transporter is a system for inorganic phosphate (Pi) acquisition in a reduced Pi environment, a cellular component important for phosphorylation of nucleic acids, lipids, sugars and proteins (Torriani, 1990).
The Tsh protein and other autotransporters

Autotransporter proteins are also widely distributed in \textit{E. coli} (Henderson \textit{et al.}, 1998). The autotransporteurs are high molecular weight proteins organized into several functional domains. The thermosensible-hemagglutinin (TSH) is part of the serine protease autotransporters of Enterobacteriaceae family. Tsh exhibits similarity in its secretion mechanism with IgA (Immunoglobulin A) and with serine proteases of Neisseria gonorrhoeae and Haemophilus influenzae (Provence and Curtiss, 1994).

Heimer \textit{et al.}, (2004) demonstrated that the autotransporter-encoding genes pic (SPATE homologue) and TSH are associated with \textit{E. coli} strains that cause acute pyelonephritis and are expressed during urinary tract infection. These determinants have been found more frequently in UPEC strains than in fecal \textit{E. coli}, suggesting a role in virulence.

Complement resistance

The complement system is one of the early stages of host defense against microorganisms. The ability to withstand the effects of complement is essentially due to the K1 capsule, certain Outer Membrane Proteins (OMP) in the outer membrane or other proteins such as ISS (Increased Serum Survival) or the lipopolysaccharides.

The K1 capsule

The \textit{E. coli} capsular antigen K1 is known to be an essential virulence factor of neonatal meningitis strains, with 80% of K1 capsule-positive \textit{E. coli} strains isolated from neonatal septicaemia or acute pyelonephritis (Kim, 2002). The K1 capsule
consists of a linear homopolymer of N-acetytneuraminic acid (NeuNAc). The biosynthesis and transport of the *E. coli* K1 capsule are mediated by a polycistronic region of 17 kb located on the chromosome which is divided into three functional regions. The region 2 is unique to each K antigen and codes for the proteins involved in the synthesis, activation and polymerization of sialic acid. The region 1 contains two genes (kps-MT); the region 3 is composed of six genes (kps-FEDUCS). The latter two regions are highly conserved across the species *E. coli*. These genes are required for the transport of the capsular polysaccharides through the cytoplasmic membrane (*kps*<sup>M</sup> and *kps*T) and their assembly on the surface of the bacterium (*kps*D and *kps*E) (Whitfield and Roberts, 1999).

**The outer membrane proteins OmpA, TraT and Iss**

The outer membrane proteins (Omp) belong to at least two types: structural proteins and porins permit the passage of small molecules. Three proteins of the outer membrane, OmpA, TraT and Iss, play a more or less important role in the resistance to serum. The first studies on the role of OmpA in the *in vivo* pathogenesis of *E. coli* for chicken were conducted in 1991. By the comparison of a K1 capsule and OmpA positive *E. coli* strain with its ompA mutant, the authors demonstrated the role of the OmpA protein in resistance to serum in vitro and *in vivo* as well as its role as a virulence factor in chicken (Weiser and Gotschlich, 1991).

**The lipopolysaccharide complex**

Lipopolysaccharide (LPS) is a key component of the outer membrane of Gram negative bacteria. It comprises three distinct regions. They are Lipid A, the oligosaccharide core, and commonly a long-chain polysaccharide, the O side chain.
Lipid A is the most conserved part of LPS. It is connected to the core part, which links it to the O repeating units. In *E. coli*, five different core structures have been described (Amor *et al.*, 2000). The O repeating units are highly polymorphic, and more than 190 serologically distinguished forms in *E. coli* are known today. The genes coding for LPS core synthesis are located at a conserved position on the *E. coli* K-12 chromosomal map (81-82 min) (Berlyn, 1998).

Several *E. coli* O antigen-encoding gene clusters have been studied, e.g. those of serotypes O7, O111, O113 and O157 (Marolda *et al.*, 1999; Wang and Reeves, 1998). They show no significant nucleotide homology between each other, with the exception of some common genes such as manC and manB. However, they contain a conserved range of predicted enzyme activities. The O6 antigen is widely distributed among pathogenic and non-pathogenic faecal *E. coli* isolates and is often found in uropathogenic *E. coli* strains. Since LPS is located on the outer surface of bacterial cells, its expression is known to be responsible for many features of the cell surface of the Gram–negative bacteria, such as resistance to detergents, hydrophobic antibiotics, organic acids, serum complement factors, adherence to eukaryotic cells. It has been suggested that some of these characteristics, especially resistance to the bactericidal effect of the complement system, are dependent on the length of the O side chain. LPS is believed to significantly contribute to virulence by protecting bacteria from the bactericidal effect of serum complement. Moreover, it has recently been reported that the K5 capsule does not contribute as much to serum resistance of *E. coli* strains as the O antigen. The lipid A is endowed with toxic properties and represents the endotoxin of Gram-negative bacteria that can be released only upon bacterial lysis. The synthesis of
several types of extracellular polysaccharides is necessary for optimal urovirulence (Bahrani et al., 2002).

Urinary tract infections (UTIs) are one of the most common bacterial infections and are predominantly caused by uropathogenic *Escherichia coli* (UPEC). *E. coli* strains belonging to 14 serogroups, including O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75 and O83, are the most frequently detected UPEC strains in a diverse range of clinical urine specimens. In the current study, the O-antigen gene clusters of *E. coli* serogroups O1, O2, O18 and O75 were characterized (Li et al., 2010).

Ellis et al., (1988) studied the relationship between serum resistance and virulence of pathogenic *E. coli* strains isolated from turkeys and showed a correlation between the serogroup of the strains and resistance to serum.

**Toxins and bacteriocins**

Toxins are prominent virulence factors of bacterial pathogens. Three toxins play a major role during UTI. They are the cytotoxic necrotizing factor 1 (CNF 1), the cytolethal distending toxin-1 (CDT-1) and α-haemolysin. CNF 1 is widely distributed in extraintestinal pathogens (Andreu et al., 1997) and belongs to a toxin family which modifies Rho, a subfamily of small GTP-binding proteins that are regulators of the actin cytoskeleton. The gene for CNF 1 is chromosomally located on different pathogenicity islands of UPEC. Eukaryotic cells intoxicated with CNF 1 exhibit membrane ruffling, formation of focal adhesions and actin stress fibers and DNA replication in absence of cell division (Aktories, 1997).
CDT-1 is a secreted protein which has the capacity to inhibit cellular proliferation by inducing an irreversible cell cycle block at the G2/M position. CDT-1 is composed of three polypeptides (CdtA, B and C) which are all required for CDT activity (Elwell et al., 2001). The direct role of the toxin in uroinfection, however, remains to be proven. The α-haemolysin is a member of the RTX toxin family, which is widely disseminated among pathogenic bacteria and widely distributed in UPEC as well as in EHEC isolates. The hly gene cluster encoding the toxin and the enzymes for its biosynthesis is located on PAIs or on plasmids. The type I secretion pathway, a posttranslational maturation and the presence of C-terminal calcium binding domain are characteristics of this pore-forming toxin (Holland et al., 1990).

Other secreted compounds, such as colicins and microcins, is also widespread among *E. coli* strains and is believed to mediate antagonistic relationships, thus contributing to competitiveness and the effective colonization of the host. Microcins are peptides of a relatively small size (1.18 to 9.00 kDa). They are considered as modified peptide antibiotics since they are synthesized as peptide precursors which are subsequently modified by other proteins. They recognize a wide range of cellular targets: colicin B17 has been shown to be an inhibitor of the DNA gyrase, colicin C7 inhibits protein synthesis, and colicin V disrupts the membrane potential (Yang and Konisky, 1984).

*FimH*-mediated cellular adhesion to mannosylated proteins is critical in the ability of uropathogenic *E. coli* (UPEC) to colonize and invade the bladder epithelium during urinary tract infection. We describe the discovery and optimization of potent
small-molecule FimH bacterial adhesion antagonists based on alpha-d-mannose 1-position anomeric glycosides using X-ray structure-guided drug design. Optimized biarylmannosides display low nanomolar binding affinity for fimH in a fluorescence polarization assay and submicromolar cellular activity in a hemagglutination (HA) functional cell assay of bacterial adhesion. X-ray crystallography demonstrates that the biphenyl moiety makes several key interactions with the outer surface of FimH including pi-pi interactions with Tyr-48 and an H-bonding electrostatic interaction with the Arg-98/Glu-50 salt bridge. Dimeric analogues linked through the biaryl ring show an impressive 8-fold increase in potency relative to monomeric matched pairs and represent the most potent fimH antagonists identified to date. The fimH antagonists described herein hold great potential for development as novel therapeutics for the effective treatment of urinary tract infections (Han et al., 2010).

*Escherichia coli* represents an incredible versatile and diverse enterobacterial species and can be subdivided into the following; (i) intestinal non-pathogenic, commensal isolates. (ii) Intestinal pathogenic isolates and (iii) extraintestinal pathogenic *E. coli* or ExPEC isolates. The presence to several putative virulence genes has been positively linked with the pathogenicity of ExPEC. *E. coli* remains one of the most frequent causes of nosocomial and community-acquired bacterial infections including urinary tract infections, enteric infections, and systemic infections in humans. ExPEC has emerged in 2000s as an important player in the resistance to antibiotics including the cephalosporins and fluoroquinolones. Most importantly among ExPEC is the increasing recognition of isolates producing "newer β-lactamases" that consists of plasmid-mediated AmpC β-lactamases (e.g., CMY), extended-spectrum β-lactamases (e.g., CTX-M), and carbapenemases (e.g., NDM). This review will highlight aspects of
virulence associated with ExPEC, provide a brief overview of plasmid-mediated resistance to β-lactams including the characteristics of the successful international sequence types such as ST38, ST131, ST405, and ST648 among ExPEC (Pitout, 2012).

**Treatment**

Overuse and abuse of antimicrobial agents has led to rapidly evolving resistance of pathogens. Appropriate administration of antibiotics to treat UTIs cannot be overemphasized. Appropriate use of antibiotics include correct indications, correct choice of antibiotic(s), and timely administration of the agent(s) with appropriate dosage and treatment duration. The pattern of antimicrobial resistance of the microorganisms causing UTI infections vary in their susceptibility to antimicrobials from place to place and from time to time (Banerjee and Padmashri, 2011).

Urinary tract infections (UTI) are frequent infections in the outpatient and hospital setting. With respect to treatment options, UTI can generally be stratified into uncomplicated and complicated / nosocomial infections. Uncomplicated UTI are represented by the acute uncomplicated cystitis and the uncomplicated pyelonephritis. They are mainly caused by *E. coli*. There are, however, also increasing resistance rates found in uncomplicated UTI, e. g., against aminopenicillins, cotrimoxazole and increasingly also fluoroquinolones. This development has called for a new evaluation of the treatment recommendations in uncomplicated UTI. As an empirical therapy for uncomplicated cystitis fosfomycin trometamol, nitrofurantoin or pivmecillinam are recommended as first-line agents. As the oral first line therapy for uncomplicated pyelonephritis fluroquinolones in high dosages are recommended. The frequent asymptomatic bacteriuria does not need to be treated, with only a few exceptions. Due
to the increasing antibiotic resistance and the emergence of multiresistant uropathogens, empirical antibiotic treatment becomes more difficult. Therefore the results of susceptibility testing should be awaited whenever possible (Wagenlehner et al., 2011).

Most stains of *E. coli*, *S. saprophyticus* and *Enterococcus* species are sensitive to nitrofurantoin. However, most species of *Proteus* and *Klebsiella* are less susceptible to this drug. Common side effects of nitrofurantoin are nausea, vomiting and diarrhea (Gupta et al., 2007).

Fosfomycin inhibits bacterial cell wall synthesis by irreversibly inactivating the enzyme pyruvyl transferase, an enzyme crucial in the synthesis of cell walls by bacteria. Fosfomycin exhibits a broad spectrum of activity against Gram positive and gram negative bacteria including *E. coli* and *P. mirabilis* (Mascaretti, 2003).

The fluoroquinolones inhibit relaxation of supercoiled DNA and cause breakage of DNA strands by inhibiting DNA gyrase and topoisomerase IV in susceptible bacteria. The fluoroquinolones that are commonly used to treat AUC are ciprofloxacin and levofloxacin. Both offer excellent coverage for Gram positive and Gram negative bacteria including Enterobacteriaceae. Common side effects of ciprofloxacin and levofloxacin are headache, nausea, diarrhea, abdominal pain and constipation (Bours et al., 2010).

β-lactam agents that can be used to treat UTI include amoxicillin-clavulanate and oral second and third generation cephalosporins. Amoxicillin inhibits bacterial cell
wall synthesis by binding to the penicillinbinding proteins. Clavulanate inhibits β-
lactamases that inactivate amoxicillin. Amoxicillin-clavulanate has a broad spectrum
which covers Gram positive bacteria, including S. saprophyticus; and Gram negative
bacteria, including E. coli and P. mirabilis. Most common side effects of amoxicillin-
clavulanate are diarrhea, nausea, vomiting and skin rash (Bours et al., 2010).

High resistance rates were observed in E. coli to ampicillin (61.4%), cefalothin
(45.5%), trimethoprim-sulfamethoxazole (38.6%), ciprofloxacin (31.8%), and
ceftriaxone (20.5%). Amikacin and nitrofurantoin were the only drugs to which >90%
of E. coli were susceptible. E. fergusonii and Serratia sp showed comparable high
resistance patterns. Thirteen strains (29.5%) of E. coli were suspected to produce
extended-spectrum beta-lactamase (ESBL) (Bours et al., 2010).

Escherichia coli was the most common uropathogen and responsible for 69.1%
of UTIs. Approximately half of E. coli isolates were resistant to ampicillin and 20.5%
to trimethoprim/sulfamethoxazole (TMP/SMX). E. coli resistance to second-generation
and third-generation cephalosporins was <4%, to aminoglycosides <8%, and to
nitrofurantoin 4.4%. Pediatric E. coli urine isolates were significantly more resistant to
ampicillin and ticarcillin and more sensitive to quinolones compared to adult E. coli
uropathogens. E. coli resistance to ampicillin and amoxicillin/clavulanic acid was
significantly higher in boys 12-23 months-old compared to girls of the same age
(Mantadakis et al., 2011).

Strains in the study patients were more resistant to antibiotics than in the control
group. Particularly large differences were noted for ciprofloxacin in E. coli (16.9% vs
7.9%) and for trimethoprim-sulfamethoxazole in *E. faecalis* (39.1% vs 24.8%). One extended spectrum β-lactamase (ESBL)-producing *E. coli* was cultured (1.3%), compared with 1.6% in the control group. No vancomycin-resistant *Enterococci* (VRE) or methicillin-resistant *Staphylococcus aureus* (MRSA) were detected (Jonsson *et al.*, 2011).

One hundred ninety-three single-patient isolates of *Escherichia coli* harboring extended-spectrum beta-lactamases (ESBL) were identified. The percentage of ESBL-producing *E. coli* among community-onset *E. coli* urine isolates increased from 0.21% in 2003 to 2.99% in 2008. One hundred seven of the ESBL producers were positive for the presence of bla (CTX-M) genes. The percentage of CTX-M-producing *E. coli* rose from 0.07% in 2003 to 1.66% in 2008. The annual percentage of ESBL *E. coli* producing CTX-Ms changed from 35% in 2003 to 64% in 2008. Genes belonging to 3 bla (CTX-M) groups: bla (CTX-M-1) group, bla (CTX-M-2) group, and bla (CTX-M) group, were detected. In addition, resistance to commonly used antimicrobial agents for community-acquired urinary tract infections was found common among CTX-M-producing *E. coli* isolates. Ertapenem and nitrofurantoin showed good in vitro activity against CTX-M producers (Qi *et al.*, 2010).

Bacteria harboring CTX-M extended-spectrum beta-lactamases (ESBLs) have been identified worldwide, with most reports coming from regions outside North America. We have identified CTX-M enzymes in 31% of ESBL-positive *Escherichia coli* isolates from our hospital and more than half (53%) of the isolates from associated long-term care facilities. Approximately 3/4 of all CTX-M-bearing isolates were from urine specimens, with a predominance of CTX-M-15. A large proportion of such
isolates were nonsusceptible to levofloxacin, trimethoprim/sulfamethoxazole, and all beta-lactam antimicrobials with the exception of the carbapenems, requiring carbapenem therapy for acute urinary tract infection or urinary tract-related sepsis. CTX-M beta-lactamases have emerged within our location, and detection of bacteria harboring these enzymes in the clinical microbiology laboratory remains problematic because molecular methods are needed for their identification (Urban et al., 2010).

A study was designed to characterize 22 non repeat extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* clinical isolates recovered from specimens originating from doctor's consultation rooms and several private and a state hospital in the Cape Town metropolitan area during 2008-2009. Characterization was done by using isoelectric focusing, PCR, sequencing of bla (CTX-M), bla (TEM), bla (SHV), and bla (OXA) as well as PCR for plasmid-mediated quinolone resistance determinants, ST131, phylogenetic groups, and plasmid replicon typing. Genetic relatedness was determined with pulsed-field gel electrophoresis using XbaI and multilocus sequencing typing. The majority of patients (17/22 [77%]) presented with urinary tract infections (UTIs) originating from the hospital setting. Thirteen (59%) of the isolates produced CTX-M-15, 7 produced CTX-M-14, and 1 isolate each produced CTX-M-3 and SHV-2, respectively. Sixteen (73%) isolates were nonsusceptible to ciprofloxacin and 8 (36%) were positive for aac (6')-Ib-cr. Overall, 10/22 (45%) of ESBL producers belonged to clonal complex ST131 that produced CTX-M-15 or CTX-M-14. Molecular characteristics of ST131 showed that this clone belonged to phylogenetic group B2. Our study illustrated that clonal complex ST131 isolates producing CTX-M-15 and CTX-M-14 had emerged as an important cause of
UTIs due to ESBL-producing *E. coli* in the Cape Town area. This is the first report to identify ST131 in ESBL-producing *E. coli* from Southern Africa (Peirano et al., 2011).

Extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* has been an emerging etiologic agent in the community acquired infections. We investigated the occurrence of ESBL producing *E. coli* isolated from patients admitted with community acquired urinary tract infection (UTI) to the hospital of the Trakya University, Turkey during 2006. Eleven single patient isolates of *E. coli* harboring ESBL were identified among 30 *E. coli* isolated from patients admitted with symptoms corresponding to upper UTI. CTX-M type ESBLs were detected in all 11 ESBL-producers by isoelectric focusing and polymerase chain reaction screening. Sequence analysis revealed CTX-M-1 in one isolate, CTX-M-3 in three isolates and CTX-M-15 in seven isolates. ESBL-producing *E. coli* isolated from community acquired UTIs are widespread in the European part of Turkey (Celik et al., 2010).

**Prevention**

The prevention of UTIs can be classified into two categories, non-antimicrobial and antimicrobial strategies.

**Herbal remedy for UTI**

Sharma *et al.*, (2009) studied seventeen Indian folklore medicinal plants to evaluate antibacterial activity of aqueous, ethanol and acetone extracts against 66 multidrug resistant isolates of major urinary tract pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* by disc diffusion method. Ethanol extract of *Zingiber officinale* and *Punica granatum* showed
strong antibacterial activity against *Escherichia coli*. Ethanol extracts of *Terminalia chebula* and *Ocimum sanctum* exhibited antibacterial activity against *Klebsiella pneumoniae*. Ethanol extract of *Cinnamomum cassia* showed maximum antibacterial activity against *Pseudomonas aeruginosa* while ethanol extract of *Azadirachta indica* and *Ocimum sanctum* exhibited antibacterial activity against *Enterococcus faecalis*. The results support the folkloric use of these plants in the treatment of urinary tract infections by the tribals of Mahakoshal region of central India. To evaluate antimicrobial nature of medicinal plant, *Emblica officinalis* fruit, *Catheranthus roseus* leaves and *Mangifera indica* seed kernel has been selected and screened for antibacterial activity against uropathogenic *E. coli*.

*Emblica officinalis* Gaertn.

*Emblica officinalis* is a medium sized tree belonging to the family Euphorbiaceae. All parts of this plant is used as a medicine. Fruit of this plant is a part of triphala, which is a most wonderful medicine for the treatment of respiratory as well as gastrointestinal infections.

**Taxonomic Position**

Kingdom : Plantae

Division : Angiospermae

Class : Dicotyledonae

Order : Geraniales

Family : Euphorbiaceae
Genus: *Emblica*

Species: *officinalis* Geartn.

**Synonym:** *Phyllanthus emblica* Linn.

**Vernacular names**

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<tr>
<td>England</td>
<td>Emblic, Indian gooseberry</td>
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</tbody>
</table>
Part used in this study

Fruit

Botanical description

Bark

Thin light grey bark exfoliating in small thin irregular flakes.

Leaves

They are simple, subsessile, closely set along the branchlets, light green having the appearance of pinnate leaves.

Flowers

They are greenish yellow, in axillary fascicles, unisexual, males numerous on short slender pedicels, females few, subsessile, ovary 3-celled.
Fruits


Geographical distribution

*Emblica officinalis* found throughout India (Rao and Siddiqui, 1964).

Traditional use

The fruits are sour, astringent, bitter, acrid, sweet, cooling, anodyne, ophthalmic, carminative, digestive, stomachic, laxative, alterant, aphrodisiac, rejuvenative, diuretic, antipyretic and tonic. They are useful in vitiated conditions of tridosha, diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematogenesis, inflammations, anemia, emaciation, hepatopathy, jaundice, strangury, diarrhoea, dysentery, hemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair (Rao and Siddiqui, 1964; Khurana et al., 1970).

Pharmacology and clinical studies

Phyllembin, isolated from the ethanolic extract of the fruit pulp has been found to potentiate the action of adrenaline *in vitro* and *in vivo*. It showed a mild depressant action on Central Nervous System and also had a spasmolytic activity. The effect of crude amla (traditionally known as amalaki rasayana) on total serum protein and its fractions was studied in rabbits. The drug had no significant effect on the levels of serum protein fractions, but it raised the total protein level and increased the body weight. The studies indicated that the increase in the body weight was due to positive
nitrogen balance. The drug was found to have only anabolic effect without affording resistance against diseases. Clinical studies were conducted to investigate the effect of crude amla in gastritis syndrome. The crude amla was given in 20 cases in a dose of 3 gms, 3 times a day for 7 days.

Safety

Rao and Siddiqui, (1964) reported that amla fruit didn’t have any side effects even after prolonged use. It was also supported by various traditional healers. Peoples in villages of Tamilnadu said that one amla fruit induces to secrete one drop of blood.

Phytochemistry

The fruits of Emblica officinalis are rich in tannins. The fruits have 28% of the total tannins distributed in the whole plant. The fruit contains two hydrolysable tannins Emblicanin A and B, which have antioxidant properties, one on hydrolysis gives gallic acid, ellagic acid and glucose wherein the other gives ellagic acid and glucose. The fruit also contains Phyllemblin (Rao and Siddiqui, 1964; Khurana et al., 1970).

Active principle: Tannins and Gallic acid

The fruit is a very rich source of vitamin C (Ghosal et al., 1996). It was a stable and potent anti-oxidant agent. Every 100g of fresh fruit provides 470 - 680mg of vitamin C. The dehydrated berry provided 2428 - 3470mg of vitamin C per 100g. Its mineral and vitamin contents include calcium, phosphorous, iron, carotene, thiamine, riboflavin, and niacin. The fruits of this tree are rich in tannin (Thakur et al., 1989).
The ethanol soluble fraction contains free sugars, D-glucose, D-fructose, D-myo-inositol. The acidic water soluble fraction contains pectin with D-galacturonic acid, D-arabinosyl, D-rhamnosyl, D-xylosyl, D-glucosyl, D-mannosyl and D-galactosyl residues (Thakur et al, 1989). The low molecular weight hydrolyzable tannins (<1,000), namely Emblicanin A and Emblicanin B, along with pedunculagin and punigluconin are the key ingredients in Emblica (Chaudhuri, 2004).

**Antibacterial, antifungal, antiviral**

The fruit ethanol extract demonstrated anti *H. pylori* activity *In Vitro* (Mehrotra et al., 2011). Acidified alcoholic extract showed the highest activity, inhibiting the growth of *M. pyogenes* var. *S. typhosa* and *S. paratyphi* at a concentration of 0.21mg/ml and that of *M. pyogenes* var. *albus; S. schotttmellari* and *S.dysenteriae* at a concentration of 0.42mg/ml.

The antimicrobial effects of extracts of *P. emblica*, against pathogenic *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas sp.*, *Klebsiella sp*, *Salmonella typhi*, *Salmonella paratyphi*, *Salmonella sp.*, *Bacillus subtilis*, *Bacillus cereus* were investigated using the agar well and discdiffusion method. Aqueous extracts of *P. emblica* were more effective than ethanolic extract producing larger zones of growth inhibition. All of natural products showed the MIC values ranged from 6.25–25.0μg/ml while the MBC values ranged from 12.5 – 100.0μg/ml (Ummey Nahor and Zakaria Ahmed, 2012).

De Britto et al., (2011) showed satisfactory activity of *P. emblica* against *Xanthomonas sp.*, *Aeromonas sp.* and *Campestris hydrophila* sp.. *Phyllanthus emblica* L Methanolic extract exhibited a significant antimicrobial activity. The Minimum
Inhibitory Concentration (MIC) exhibited by Phyllanthus emblica L methanolic extract against the tested organisms ranges between 0.261 and 0.342.

The aqueous fruit extracts of *E. officinalis* showed the antibacterial activity against all the five test bacterial strains which supports the potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. It was clear from the present results that the aqueous fruit extracts of *E. officinalis* exhibited pronounced activity against the five tested bacteria namely *Bacillus* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Proteus* sp., and *Streptococcus* sp. (Mir *et al.*, 2012).

Saheb *et al.*, (2010) assessed the antibacterial activity in tannins isolated from the leaves and fruits of *E. officinalis*. The aqueous leaf extracts of *P. niruri* shows inhibitory action towards *Lactobacillus* sp. only and it does not show any inhibition on other test bacteria cultures. Manas *et al.*, (2012) revealed that the methanolic extracts of various parts of *Phyllanthus niruri* have antibacterial activity against five bacterial strains - *E. cloacae*, *S. aureus*, *P. aeruginosa*, *E. coli* and *S. viridians* and two fungal strains - *A. niger* and *T. viridae*. The chloroform soluble fraction of the methanolic extract of *E. officinalis* displayed significant antimicrobial activity against some gram- positive and gram- negative pathogenic bacteria with a strong cytotoxicity having a LC$_{50}$ (Lethal Concentration) of $10.257 \pm 0.770$ microg mL $^{-1}$ (Rahman *et al.*, 2009).
The aqueous extracts of the fruit pulp of *E. officinalis* were evaluated by Vijayalakshmi *et al.*, (2007) for antimicrobial activity against gram- positive bacteria *Staphylococcus aureus*, gram- negative bacteria *Escherichia coli* and fungal strains of Candida species by using agar cup plate method. The extracts showed a different degree of activity against pathogenic microbes. Aqueous infusion and decoction of *E. officinalis* exhibited 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 *et al.*, 2007).

Saini *et al.*, (2008) evaluated the effect of *E. officinalis* administration on the vulnerability of experimental mice to respiratory tract infection induced by *K. pneumoniae*. These results suggest that dietary supplementation with *E. officinalis* protects against bacterial colonization of lungs on long-term feeding in an experimental model. Further studies need to be conducted to understand the actual mechanism. Thaweboon *et al.*, (2011) demonstrated that *E. officinalis* ethanolic extract interferes with the adhesion of *C. albicans* to BECs (human buccal epithelial cells) and denture acrylic surfaces *in vitro*. Promising antiplasmodial activity was found in the extracts from *E. officinalis* leaf. They were also found to be active against Chloroquine-resistant strains. These results demonstrate that extracts of *E. officinalis* may serve as antimalarial agents even in their crude form. Bagavan *et al.*, (2011) and Pinmai *et al.* (2010) revealed that *in vivo* antiplasmodial activity with good suppression activity ranged from 53.40% to 69.46%.

Polyphenolic compound isolated from *E. officinalis* might exert anti-herpes simplex virus (HSV) activity both by inactivating extracellular viral particles and by
inhibiting the viral biosynthesis in host cells. These results warrant further studies on its antiviral mechanisms and suggest that it might be a candidate for HSV therapy (Xiang et al., 2011). *E. officinalis* definitely possesses potent antimicrobial activities, thus serving as an important platform for the development of inexpensive, safe and effective medicines (Kumar et al., 2011).

Experiments conducted at the Niwa Institute of Immunology in Japan have shown Amla to be a potent scavenger of free radicals. The studies showed that Amla preparations contained high levels of the free-radical scavenger, superoxide dimutase (SOD), in the experimental subjects. In Indonesia, the pulp of the fruit is smeared on the head to dispel headache and dizziness caused by excessive heat. Amla is mixed with buttermilk for anointing and "cooling" the head (Treadway, 1994).

Skin lightening agents have been widely used to both lighten and depigment the skin in the Asia, Far East and Middle East countries, whereas in the European market products tend to be employed for age spots and freckles. The effectiveness of a standardized antioxidant fraction of *Phyllanthus emblica* fruits as a skin lightener and also as an antioxidant was proven (Chaudhuri, 2004). *Phyllanthus emblica* L. fruit purified phenolics demonstrated antioxidant activity *in vitro* (Luo et al., 2011). The fruit exhibited wound healing activity via improvement of collagen function and enhancing antioxidant capacity (Sumitra et al., 2009). It protected against gastric ulcer via its antioxidant and cytoprotective activity (Bandyopadhyay et al., 2000; Chatterjee et al., 2012). Gallic acid enriched extract exhibited healing property on gastric ulcer via increasing PG E2 and proangiogenesis factors, enhancing endothelial NOS (eNOS), and regulation of pro-inflammatory and anti-inflammatory cytokines and antioxidant activity (Chatterjee et al., 2012; Bandyopadhyay et al., 2011).
**Mangifera indica** Linn

**Introduction**

Mango belongs to the genus *Mangifera* of the family Anacardiaceae. The genus *Mangifera* contains several species that bear edible fruit. Most of the fruit trees that are commonly known as mangos belong to the species *Mangifera indica*. Mango has become naturalized and adapted throughout tropics and subtropics. There are over 1000 named mango varieties throughout the world, which is a testament of their value to humankind. Seed kernel of mango is used as cure for chronic and acute diarrhoea throughout the world.

**Taxonomic position (Bentham and Hooker, 1862-1893)**

- **Kingdom**: Plant kingdom
- **Subkingdom**: Dicotyledones
- **Class**: Polypetalae
- **Sub class**: Rosidae
- **Series**: Disciflorae
- **Order**: Sapindales
- **Family**: Anacardiaceae
- **Genus**: Mangifera
- **Species**: Mangifera indica Linn
**Botanical Name**

*Mangifera indica*

**Names in regional languages**

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**Name in various systems of Medicine**

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Habitat

*M. indica* grows from sea level to 1200 meter (3950 ft) in tropical latitudes. Most of the commercial varieties grow below 600 meter (1950 ft). It is Cultivated in many districts of Tamilnadu. Mango is now cultivated throughout tropical and subtropical region for commercial fruit production, as a garden tree, and as a shade tree for stock. All mango varieties were introduced from other parts of the world to Pacific region (www.traditionaltree.org).

Habit

Source taxons of mango are long-lived, evergreen trees that can reach to a height of about 15–30 m (50–100 ft). Most cultivated mango trees grow to a height between 3 and 10 m (10–33 ft) tall when fully mature, depending on the variety and the amount of pruning. Wild, non-cultivated seedling trees often reach 15 m (50 ft) when found in favorable climates, and they can reach 30 m (100 ft) in forest. The trees can live for over 100 years and develop trunk girths of over 4 m (13 ft).

Chemical constituents

Mango plant in general contains 2-octene, alanine, alpha-phellandrene, alpha-pinene, ambolic-acid, ambonic-acid, arginine, ascorbic-acid, beta-carotene beta-pinene, carotenoids, furfurol, gaba, gallic-acid, gallotannic-acid, geraniol, histidine, isoleucine, isomangiferolic-acid, kaempferol, limonene, linoleic-acid, mangiferic-acid, mangiferine, mangiferol, mangiferolic-acid, myristic-acid, neo-beta-carotene-b, neo-
beta-carotene-u, neoxanthophyll, nerol, neryl-acetate, oleic-acid, oxalic-acid, p-coumaric-acid, palmitic-acid, palmitoleic-acid, pantothenic-acid, peroxidase, phenylalanine, phytn, proline, quercetin, xanthophyll (http://www.rain-tree.com/mango.htm) Xanthoe C-glycoside homomangiferin.

**Medicinal properties** (Morton, 1987)

*Mangifera indica* and its parts have been used for various purposes. In India, fruit is used as a laxative and diuretic. Fruit sap has been used to treat pain of bee and scorpion stings. Fruits are eaten as a kidney tonic and to cure headaches. Half-ripe fruit eaten with salt and honey is for a treatment of gastro-intestinal disorders, bilious disorders, blood disorders and scurvy. Ripe mangos are rich source of vitamin A and are used to treat vitamin A deficiencies like night blindness. Green fruits are considered anticholeric, antidysmenorrheic, anti scorburtic, astringent, and diaphoretic. Ripe fruits are considered diuretic, laxative, and unguent. Extracts of unripe fruits and bark, stems and leaves have antibiotic activity on gram positive and gram negative bacteria. Ripe mangos are said to contain anti-fungal properties.

Seeds are used to cure asthma. Seed kernels are used to cure chronic diarrhoea, to expell tape worms and other worms in ulcers (Warrier, 1994 and Kurian, 1995). Powdered seed kernel is used as antihelminthic. Traditionally tribal people of India eat mango seed kernel in roasted form during starvation as it is rich in Starch (Rukmini and Vijayaraghavan ,1984). Hence it was assumed to be suitable for human consumption (Ramteke et al., 1999). The kernel powder is used as astringent in bleeding piles (Keher and Chanda, 1946). The seed of Mangifera indica is reported in traditional medicine as a cure vomiting, dysentery and burning (Krithikar and Basu, 1984). In villages Mangifera indica seed kernel is used along with honey to treat helminthic infections.
Mango seed kernel decoction and powder (not tannin-free) are used as vermifuges and as astringents in diarrhoea, hemorrhages and bleeding hemorrhoids. The seeds are anthelmintic, antiasthmatic, antimenorrhagic and antidysenteric. Paste is made from mango seed (Kernel), honey and camphor and applied over vagina in order to make the vagina contracted and firm (Sharma, 2003).

In Nigeria, mango seed kernel is processed into powder and substituted for wheat flour in biscuits (Arogba, 1999). In South Asian folk medicine, rheumatism and diphtheria is treated using astringent made from bark. In Samoa, a bark infusion has been a traditional remedy for mouth infections in children. Immature mango leaves are cooked and eaten in Indonesia and Philippines. In Tonga, infusions of mango leaves and orange are used to treat relapse sickness. In Caribbean islands, leaf decoction is taken as a remedy for various ailments such as diarrhoea, fever, chest complaints, diabetes, hypertension and other ills (Morton, 1987 and www.hort.purdue/mango).

In Ayurveda and Siddha, dried mango flowers are used to cure dysentery, diarrhoea and inflammation of urinary tract. Seed kernel is used to treat diarrhoea, dysentery. In Unani, mango are used for strengthening nervous and blood systems, removing toxins from blood and treating anaemia (Nadkarni, 1987; Warrier, 1994; Annonymous, 1962).
Table 3.2

Ethnomedical uses of *Mangifera indica* parts

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<th>Part</th>
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<td>Dried Seed</td>
<td>Dandruff</td>
<td>John, 1984</td>
</tr>
<tr>
<td>3</td>
<td>India</td>
<td>Kernel</td>
<td>Antihelminthic, aphrodisiac, laxative, tonic</td>
<td>Sharma <em>et al.</em>, 1971</td>
</tr>
<tr>
<td>4</td>
<td>Nepal</td>
<td>Seed</td>
<td>Asthma</td>
<td>Suwal, 1970</td>
</tr>
<tr>
<td>5</td>
<td>Nepal</td>
<td>Seed</td>
<td>Diarrhoea</td>
<td>Bhattarai, 1992</td>
</tr>
</tbody>
</table>

**Parts Used**

In Siddha system of medicine root bark, stem bark, leaf, flower, immature fruit, seed and gum were used as medicine. In Ayurvedha bark, leaf, flowers, fruit and seed kernel were used as medicine (Nadkarni, 1987; Warrier, 1998).

**Pharmacological activities**

Seed kernel of *Mangifera indica* has antioxidant and antidiarrhoeal activity. Yean and Philip (2004) reported that seed kernel of *Mangifera indica* had a higher antioxidant activity. Seeds of *Mangifera indica* have been used for its anti-diarrhoeal activity in Indian traditional medicine (Sairam *et al.*, 2003). Mango seed kernel is a promising source of food additive. It enhances oxidative stability of food and used as a food preservator (Toshihidi, 2000). Dried mango flowers, containing 15% tannin, served as astringents in cases of diarrhea, chronic dysentery, catarrh of the bladder and chronic urethritis resulting from gonorrhoea. The bark contains mangiferin and is astringent and employed against rheumatism and diphtheria in India. Toxic compounds are not detected in mango seed kernel. They seems to be a safe source of antioxidant (Rukmini and
Vijayaraghavan, 1984). Mangifera indica has been used along with other classical traditional antidiarrhoeal drugs like Holarrhena antidysenterica and Oxoylum indicum (Singh and Chathurvedi, 1981). Mangifera indica seed kernel is used for the treatment of dysentery (Singh, 1986), diarrhoea (Ponce et al., 1994).

Aqueous extract inhibited the growth of Streptococcus aureus and Proteus vulgaris (Sairam et al., 2003). Ethanolic extract of Mangifera indica seed kernel showed good and effective activity against both gram positive and gram negative bacteria especially pathogenic serogroups of Escherichia coli, Salomonella enteritis, Shigella flexneri, Klebsiella, Yersinia enterocolitica, Vibrio sp. Campylobacter sp. S. aureus etc.(Toshihidi, 2000). Ascardicidal activity of Mangifera indica seed kernel was showed by Feroz et al., (1982). Mangifera indica seed kernel showed an anti inflammatory activity (Das et al., 1989).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Country</th>
<th>Part</th>
<th>Active against</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>Kernel</td>
<td>Agrobacterium sp., Proteus sp., Pseudomonas sp., S.flexneri, E. coil, S.typhi, S.enteritidis, S.dysenteriae</td>
<td>Das et al., 1989</td>
</tr>
<tr>
<td>2</td>
<td>Puerto Rico</td>
<td>Dried leaf</td>
<td>Mycobacterium tuberculosis</td>
<td>Frame et al., 1998</td>
</tr>
<tr>
<td>3</td>
<td>Zaire</td>
<td>Dried stem bark</td>
<td>S. aureus, Klebsiella sp.</td>
<td>Muanza et al., 1994</td>
</tr>
<tr>
<td>4</td>
<td>Zaire</td>
<td>Dried stem bark</td>
<td>S. aureus, Klebsiella sp., Salmonella enteritidis, Escherichia coli</td>
<td>Lutete et al., 1994</td>
</tr>
</tbody>
</table>
Table 3.4
Antiviral, Antifungal and Antiparasitic activities of *Mangifera indica*

<table>
<thead>
<tr>
<th>S. No</th>
<th>Country</th>
<th>Part</th>
<th>Active against</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>Kernel</td>
<td>Antifungal - <em>Trichophyton mentagrophytes</em>, <em>Candida lutea</em>, <em>Candida albicans</em></td>
<td>Das <em>et al.</em>, 1989</td>
</tr>
<tr>
<td>2</td>
<td>India</td>
<td>Dried seed</td>
<td>Anti ascardial activity – <em>Ascaris lumbricoides</em></td>
<td>Feroz <em>et al.</em>, 1982</td>
</tr>
</tbody>
</table>

Table 3.5
Other activities of *Mangifera indica*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Country</th>
<th>Part</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>India</td>
<td>Kernel</td>
<td>Antihelminthic</td>
<td>Sharma <em>et al.</em>, 1971</td>
</tr>
<tr>
<td>2</td>
<td>India</td>
<td>Kernel</td>
<td>Antiinflammatory</td>
<td>Das <em>et al.</em>, 1989</td>
</tr>
<tr>
<td>3</td>
<td>India</td>
<td>Kernel</td>
<td>Cholestrol level decrease</td>
<td>Anila and Vijayalakshmi, 2003</td>
</tr>
</tbody>
</table>

*Aegle marmelos* (L.) Corr.

Indian Medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments (Biswas *et al.*, 2002; Chatopadhyay *et al.*, 2004). Bael (*Aegle marmelos* (L.) Corr.) is another Indian medicinal plant; which has enormous traditional values against various diseases and many bioactive compounds have been isolated from this plant (Badam *et al.*, 2002; Gupta and Tondon, 2004). *A. marmelos* is a native plant of India. *A. marmelos* belongs to Rutaceae family and commonly known as wood apple. In India, *A. marmelos* is grown as a temple garden plant and the leaves are used to pray Lord Shiva. *A. marmelos* fruit is an important medicinal plant with several ethnomedicinal applications in traditional and folk medicinal systems. Traditionally, *A. marmelos* is used in the treatment of diarrhoea and dysentery (Dinesh *et al.*, 2011)

**Taxonomic Position**

Kingdom : Plantae
Division : Magnoliophyta
Order : Sapindales
Class : Magnoliopsida
Family : Rutaceae
Sub family : Aurantioideae
Genus : Aegle
Species : A. marmelos

Names of Aegle marmelos in different languages

Common name: Wood Apple
Latin : Aegle marmelos
English : Wood/Stone apple, Bengal Quince, Indian Quince
Vietnamese : Mbau Nau, Trai Mam
Nepali : Bel, Gudu
Sino-Tibetan : Toum
Khmer : Bnau
Javanese : Modjo
French : Oranger du Malabar
Burmese : Ohshit, opesheet
Indonesian : Mojo tree
Malay : Pokok Maja Batu
Thai : Mapin, Matum, Tum
Sanskrit : Shreephal, Bilva, Bilwa
Old Hindi : Sir Phal
Bengali : Bel, Shreefal
Marathi : Kaveeth
Tamil : Vilva Maram, Vilva Pazham
Telugu : Maredu
Urdu : Bel
Gujarati : Bilaphjal, Bilinuphal, Billi, Bilivaphal, Bil.
Kannad : Bela, Bilva, Bilvapatara
Malayalam : Maviladu, Kulakam, Koovalum

**Plant Description**

*Aegle marmelos* is a medicinal plant which is fairly large sized deciduous and glabrous tree with auxillary spines and usually trifoliate leaves belonging to the family Rutaceae (Prakash and Prasad., 1969).

**Tree**

*A. marmelos* is a slow-growing, medium sized tree, 25 to 30 feet tall.

**Stem**

The stem is short, thick, soft, flaking bark, and spreading, sometimes spiny branches, the lower ones drooping. Young suckers bear many stiff, straight spines. There are sharp, axial one inch long spikes on this tree. The leaflets are oval or lancet shaped, 4-10 cm long, 2-5 cm wide.

**Leaves**

Leaves composed of 3 to 5 leaflets in it. The lateral leaflets are without petiole and the terminal one has a long one. The petiole is 1 to 2.5 inch long. Mature leaves emit a peculiar fragrance when bruised.
Flowers

Flowers occurs in clusters of 4 to 7 along the young branchlets, have 4 recurved, fleshy petals. The flowers are greenish white in color with a peculiar fragrant. Flowering occurs during the month of May and June.

Root

The roots are yellowish-white to light brown often with an additional light-purple or rose tint. Their surface shows the information of numerous lenticels in several longitudinal series. The root bark has a firm leathery texture and is bitter, pungent and aromatic. The wood of the root is mildly fragrant and slightly sweetish.

Bark

It is cream yellow or grayish or yellowish brown, firm or compact, leathery and slightly aromatic. It is about 12mm thick exfoliating irregular flakes soft and corky. It has compactively soft surface, free of warts thorough it is a profusely laticellate.

Flowers

Flowers are greenish white, sweetly scented, 5 meros, bi sexual 2.5 cm across, calyx tube capsular to 5 mm; Lobes 4 or 5 triangular, petioles 5, white, oblong, sub equal, 1× .6 cm fleshly, spreading. Disc obscure, stamens α, Ca 50 filaments to 3mm basely sub connate; anthers oblong to 4 cm ovary ovoid, > 10 celled, sigma subssesible oblong. Berry ovoid, 8×6 cm, woody, seeds and oblong to 8×4 to 7 along the young branchlets have a 4 recurred, freshly fetal’s, green outside, yellowish inside and 50 or more greenish yellow statements.
**Fruit**

Fruit is spherical or oval in shape with a diameter of 2 to 4 inch. Shell is thin, hard and woody in nature. It is greenish when unripe and upon ripening it turns into yellowish color. The pulp of the fruit has 8 to 15 segments. The pulp is yellow, soft, pasty, sweet, resinous and fragrant. Fruition occurs in the month of May and June. The peel of the fruit which is a very hard shell and green to brown in colour depends on ripening stage. The appearance of yellow or orange edible pulp is like a boiled pumpkin, possesses a slightly sweet taste and a characteristic floral, terpene-like aroma, very fragrant and pleasantly flavored.

**Seeds**

The seeds are embedded in the pulp. The seeds are small (nearly 1 cm in length), hard, flattened-oblong, bearing woolly hairs and each enclosed in a sac of adhesive. Seeds are surrounded by slimy transparent mucilage (Brijesh et al., 2009).

**Habitat**

*A. marmelos* is a subtropical plant and grows up to an altitude of 1,200 m altitude from sea level. It grows well in the dry forests on hilly and plain areas. *A. marmelos* is a widely distributed plant and found in India, Ceylon, China, Nepal, Sri Lanka, Myanmar, Pakistan, Bangladesh, Nepal, Vietnam, Laos, Cambodia, Thailand, Indonesia, Malaysia, Tibet, Sri Lanka, Java, Philippines and Fiji. In India it found in Sub-Himalayan tracts from Jhelum eastwards to West Bengal, in central and south India. It found almost in all the states of India (Brijesh *et al.*, 2009). It is grown throughout India as well as in Sri Lanka, Pakistan, Bangladesh, Burma, Thailand, and most of the Southeast Asian countries (Singh and Roy, 1984).
Traditional use of *Aegle marmelos*

*A. marmelos* is extensively described in the Vedic literature for the treatment of various diseases. *A. marmelos* is traditionally used to treat jaundice, constipation, chronic diarrhoea, dysentery, stomachache, fever, asthma, inflammations, febrile delirium, acute bronchitis, snakebite, abdominal discomfort, acidity, burning sensation, epilepsy, indigestion, leprosy, myalgia, smallpox, spermatorrhoea, leucoderma, eye disorders, ulcers, mental illnesses, nausea, sores, swelling, thirst, thyroid disorders, tumors, ulcers and upper respiratory tract infections (Daswani *et al.*, 2009).

**Chemical Constituents**

Various phytoconstituents have been isolated from the leaves are Skimmianine, Aegeline, Lupeol, Cineol, Citral, Citronella, Cuminaldehyde, Eugenol, Marmesinine. Different organic extracts of the leaves of *A. marmelos* have been reported to possess alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids and steroids (Maity *et al.*, 2009).

**Various proved therapeutic values of *Aegle marmelos***

The different parts of Bael are used for various therapeutic purposes, such as for treatment of Asthma, Anaemia, Fractures, Healing of Wounds, Swollen Joints, High Blood Pressure, Jaundice, Diarrhoea Healthy Mind and Brain Typhoid Troubles during Pregnancy (Saswati Parichha 2004). *Aegle marmelos* has been used as a herbal medicine for the management of diabetes mellitus in Ayurvedic, Unani and Siddha systems of medicine in India (Kar *et al.*, 2003), Bangladesh (Lampronti *et al.*, 2003) and SriLanka (Karunanayake *et al.*, 1984). The main usage of the parts of this tree is for medicinal purposes. The leaves are made into a poultice and used in the treatment of
ophthalmia. The leaf part of the plants have been claimed to be used for the treatment of inflammation, asthma, hypoglycemia, febrifuge, hepatitis and analgesic. Leaf poultice is applied to inflammation; with black pepper for edema, constipation and jaundice.

Leaves of this plant used to cause infertility-abortion in women. Recently, the plant is screened for its medicinal properties by scientific techniques and reported for various medicinal properties. The present review aims to document the morphology, distribution, phytochemistry and medicinal properties of *A. marmelos* and its future prospects for the further scientific investigation for the development of effective therapeutic compounds.

*A. marmelos* is extensively described in the Vedic literature for the treatment of various diseases. A. marmelos is traditionally used to treat jaundice, constipation, chronic diarrhea, dysentery, stomachache, stomachic, fever, asthma, inflammations, febrile delirium, acute bronchitis, snakebite, abdominal discomfort, acidity, burning sensation, epilepsy, indigestion, leprosy, myalgia, smallpox, spermatorrhoea, leucoderma, eye disorders, ulcers, mental illnesses, nausea, sores, swelling, thirst, thyroid disorders, tumors, ulcers and upper respiratory tract infections.

**Anti diabetic Activity**

Aqueous extract of *Aegle marmelos* leaves, was evaluated for hypoglycemic and antioxidant effect by Upadhya *et al.*, (2004) by using alloxan induced diabetes in male albino rats and proposed AML may be useful in the long-term management of diabetes. Antidiabetic potential of the leaves and callus of *A. marmelos* was reported in streptozotocin induced diabetic rabbits. All the extracts reduced the blood sugar level in
streptozotozin diabetic rabbits, however, among the various extracts, the methanol extracts of the leaf and callus brought about the maximum anti-diabetic effect. The anti-diabetic activity of the leaves of Aegle marmelos was reported in alloxan diabetic rats. The methanolic extract (120 mg/kg body weight, ip) of the leaves of Aegle marmelos reduces the blood sugar level. Reduction in blood sugar could be seen from 6th day after continuous administration of the extract and on 12th day sugar levels were found to be reduced by 54%.

Kuttan & Sabu (2004) studied on leaf extract of Aegle Marmelos on Alloxane induced diabetes and reported that used extract was enough capable to reduce oxidative stress by scavenging lipid peroxidation and enhancing certain Anti oxidant levels which causes lowering of elevated blood glucose level. Beside of all above cited work, Hema & Lalithakumari (1999) had presented tremendous results of Aegle marmelos and documented its hypoglycemic action along with other pharmacological actions on molecular level.

**Hepatoprotective activity**

Singanan et al., (2007) worked on Aegle marmelos leaf extract on alcohol induced liver injury in albino rats and presented data of excellent hepatoprotective effects.

**Analgesic, anti-inflammatory and antipyretic Activity**

Arul et al., (2005) presented anti-inflammatory, antipyretic & analgesic properties of serial extract of leaves of Aegle Marmelos, and presented that most of the extract caused a significant inhibition of the carrageenan-induced paw oedema and
cotton-pellet granuloma in rats. The extracts also produced marked analgesic activity by reduction the early and late phases of paw licking in mice. A significant reduction in hyperpyrexia in rats was also produced by the most of the extracts. Similarly, Ghangale (2008) also evaluated aqueous extract of Aegle mannelos for anti inflammatory activity by using rat paw oedema model and proposed that Aegle marmelos possesses anti-inflammatory activity. Shankharananth (2007), demonstrated that methanolic extract of leaves of Aegle marmelos at a dose level of 200 and 300 mg/ kg show significant analgesic activity on acetic acid induced writhing and tail flick test in mice.

**Anti microbial Activity**

Patil (2009) reported the antifungal activity of ethanol extract of the Aegle marmelos leaves including antidiarrhoeal, and antimicrobial, activities. Rana (1997) evaluated anti fungal activity of essential oils isolated from the leaves of Bael using spore germination assay. The oil exhibited variable efficacy against different fungal isolates and 100% inhibition of spore germination of all the fungi tested was observed at 500ppm. They proposed that essential oil from bael leaves may interfere with the \( \text{Ca}^{2+} \)-dipicolonic acid metabolism pathway and possibly inhibit the spore formation. Pitre and Srivastava (1987), demonstrate the antifungal activity of ethanolic root extract against *Aspergillus fumiganus* and *Trichphyton mentagrophytes*.

Antimicrobial Activity *A. marmelos* has been traditionally used for the treatment of various infectious diseases and been extensible reported to inhibit the broad range of pathogenic microorganisms. Many in vitro studies proved the antimicrobial potential of *A. marmelos* extracts towards the pathogenic microorganisms including bacteria and fungi. The aqueous, petroleum ether and ethanol extract of the
leaves of *Aegle marmelos* exhibited efficient antimicrobial activity against *Escherichia coli, Streptococcus pneumoniae, Salmonella typhi, Klebsiella pneumoniae* and *Proteus vulgaris*. The ethanolic extract shows activity against *Penicillium chrysogenum* and the petroleum ether and aqueous extract shows activity against *Fusarium oxysporum* (Balakrishnan *et al.*, 2006).

The antimicrobial activity of the leaves of *Aegle marmelos* was reported against multi resistant strains of *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antimicrobial activity against gram-negative strains was higher than that of gram positive strains. The antifungal activity of the leaves of *Aegle marmelos* was reported against clinical isolates of dermatophytes (Rajan *et al.*, 2011 and Ulahannan *et al.*, 2008).

The antimicrobial activity was checked against *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli, Salmonella paratyphi A* and *Salmonella paratyphi B*. The methanol extract showed significantly high activity against above mentioned bacteria than that of the other extracts. The antibacterial activity of the leaves of Aegle marmelos was reported. The antibacterial activity of the different extracts was evaluated by agar well diffusion method. The hexane, cold methanol, hot methanol and ciproflaxacine extracts showed high antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Micrococcus luteus, Enterococcus faecalis* and *Streptococcus faecalis* (Rana *et al.*, 1997; Sudharameshwari and Radhika, 2007; Suresh *et al.*, 2009).
The antibacterial activity of the leaves of *A. marmelos* Methanol extract showed high antibacterial activity against the test organisms. The antibacterial activity of the various solvent extracts of the *Aegle marmelos* leaves was reported. The antimicrobial activity of the various solvent extracts was screened by modified disc diffusion assay. Different extracts showed antibacterial activity against *Micrococcus glutamicus*, *Streptococcus faecalis*, *Staphylococcus aureus*, *S. pyogenes*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *E. coli* and *Pseudomonas denitrificans*. Petroleum ether extract did not resulted in any activity while ethanol and chloroform extract exhibits maximum activity (Malviya *et al.*, 2012).

**Radioprotective Activity**

Radioprotective effect of *Aegle marmelos* extract was studied by Jagetia and Venkatesh (2005) by exposing to different doses of gamma-radiation in mice and found that oral administration of extract resulted in an increase in radiation tolerance by 1.6 Gy. Again, Jagetia *et al.*, (2006), studied effects of plant extract on the peripheral blood and small intestine of Swiss albino mice. They exposed the animals to gamma radiation and data were collected against radiation-induced changes in the peripheral blood, spleen colony forming units, and intestinal mucosa, reported that *Aegle marmelos* extract significantly reduces the deleterious effect of radiation in intestine and bone marrow of mouse.

**Antispermatogenic Activity**

Pramanik *et al.*, (1999) reported antispermatogenic acitivity of ethanolic extract of *Aegle marmelos* leaves in rats. Sharma *et al.*, (2009), studied the effect of ethanol extracts of leaves of *A. marmelos* for their *in vitro* effect on sperm motility and was
suggested that the extracts had a considerable effect on the motility of sperm. It was also proposed that an increase in concentration of the extracts decreased the motility of sperms.

**Antiulcer Activity**

Goel (1997) reported that oral; administration of pyranocoumarin isolated from the seeds of *Aegle marmelos* Correa, showed significant protection against pylorus-ligated and aspirin-induced gastric ulcers in rats and cold restraint stress-induced gastric ulcers in rats and guinea pigs. Dhuley (2007), reported that pretreatment of rats with unripe bael fruit extract produce a significant inhibition of absolute ethanol induced gastric mucosal damage.

**Anti thyroid Activity**

Panda and Kar (2006), isolated, Scopoletin (7-hydroxy-6-methoxy coumarin) from Aegle marmelos leaves and evaluate for its potential to regulate hyperthyroidism. It was observed that scopoletin (at 1.00 mg / kg, p.o. for 7 days) to levo-thyroxine treated animals, decreased serum thyroid hormones level. It was also proved that the scopoletin have superior therapeutic activity than the standard antithyroid drug, propylthiouracil.

**Toxicity Studies**

Total alcoholic, total aqueous, whole aqueous and methanolic extracts were collected from the leaves of *A. marmelos* by the Veerappan *et al.*, (2007) and studied in experimental rats for their toxicity. No histopathological changes were found when extracts of *A. marmelos* were administered intraperitoneally for 14 days successively at
the dose of 50 mg/kg body wt. The collected data demonstrate that the extracts of the leaves of *A. marmelos* have a high margin of drug safety.

**Other reported medicinal values**

The antidiarrhoeal effect of aqueous extract of *Aegle marmelos* fruit have been reported by effecting outer membrane protein C of *Enteropathogenic Escherichia coli* (Subramaniya *et al.*, 2009). Besides these activities, Insecticidal activity (Kumar *et al.*, 2008), Anti-lipid peroxidative activity (Kamalakkannan and Prince, 2003), Antioxidant property (Vimal and Devaki, 2004).

**Catheranthes roseus**

*Catharanthus roseus* L.(G.) Don. (Periwinkle) belongs to family Apocyanceae and is found abundantly all over world. It is short – lived perennial with dark green and glossy leaves. Pharmacological studies have revealed that *C. roseus* contain more than 70 different type of alkaloids (indole alkaloids) and chemotherapeutic agents that are effective in treating various type of cancers-breast cancer, lung cancer, uterine cancer, melanomas, hodgkin’s and non-hodgkin’s lymphoma. Traditionally, *Catharanthus roseus* L. (G.) Don has been used in folk medicine to treat diabetes and high blood pressure. As antidiabetic remedy, it was believed to promote insulin production and increase utilization of sugars from food. Its diuretic action, alleviate high blood pressure. However, in modern medicine alkaloids and chemotherapeutic agents from *C. roseus* are known for their anticancer, pain-relieving properties. The anticancer drugs vincristine and vinblastine are synthesized from alkaloids of *Catharanthus roseus* L. (G.) Don. The plant is also known for its antihypertensive and antispasmodic
properties due to presence of alkaloids like ajamalicine, serpentine and reserpine. The root bark contains the alkaloid alstonine which has been used traditionally for its calming effect and its ability to reduce blood pressure. There is little doubt in the importance of *Catharanthus roseus* as medicinal plant. The vast collection of literature and publications pertaining to this plant of high medicinal value very well illustrates this fact. About 295 patents dealing with the plant and its products have been reported (Kratika and Sharmita, 2013). Ironically, till date, very little studies have been done on the antimicrobial properties.

**Taxonomic position**

Class: Equisetopsida  
Subclass: Magnoliidae  
Superorder: Asteranae  
Order: Gentianales  
Family: Apocynaceae  
Genus: Catharanthus

**Botanical Description**

It is an erect, smooth or slightly hairy, simple or slightly branched plant, 30 to 50 centimeters high.

Stems are somewhat woody.

Leaves are oblong, 4 to 7 centimeters long, rounded at tip, pointed at base.

Flowers are white, pink, or red, or variegated white and red, 3.5 cm to 5 centimeters across, borne in the axils of the leaves. Calyx-lobes are green and very slender about 4
millimeters long. Corolla-tube is slender, 2.5 to 3 centimeters long, and pale green; the limb is spreading with obliquely obovate lobes 1.7 to 2.5 centimeters wide.

Fruit is a hairy and cylindric follicle, 2 to 3 centimeters long.

**Distribution**

It is introduced as an ornamental. It is a native of tropical America. This plant flowers throughout the year.

**Chemical Constituents**

Leaves of this plant yield a volatile oil containing aldehyde, sesquiterpenes, furfural, sulphur-containing compounds, lochnerol, vincamine, vinpocetin (ethyl aponvineminate), vincarosin. Plant yields an amorphous alkaloid, vincarosin. Compounds identified: Alkaloids (vincristine, vinblastine, ibogaine, yohimbine, raubasine), flavonoids (hirsutidin). Plant yields more than 100 monoterpenoid indole alkaloids. Leaves and stems yield dimeric alkaloids vincristine and vinblastine. Roots yield ajmalicine and serpentine.

**Properties**

Leaves are vomitive. Leaf alkaloids considered anti-cancer. Roots are purgative, vermifuge, depurative, hemostatic considered antibacterial, antifungal and antiviral.

**Parts utilized**

Leaves and whole plants.
**Folkloric uses**

Philippines: decoction of leaves used in diabetes. Decoction of young leaves used for stomach cramps. Infusion of leaves used for treating menorrhagia. Crude leaf extract has anticancer activity.

Madagascar: the bitter and astringent leaves used as vomitive.

Orissa: juice of leaves used as application to wasp stings.

Mauritius: infusion of leaves used for indigestion and dyspepsia.

India: juice of leaves used for bee stings and diabetes.

West Indies: used for diabetes.

Nigeria: used for diabetes.

**Pharmacology**

Study on the leaf juice of *C. roseus* showed a dose-dependent lowering of blood glucose in both normal and diabetic rabbits comparable to the standard drug, glibenclamide. The mechanism of action was probably through enhanced secretion of insulin from the β-cells (Srinivas *et al.*, 2003).

The anti-cancer drugs, vincristine and vinblastine, are derived from the alkaloids of periwinkle. The alkaloid has growth inhibition effects to some human tumors. Vinblastine is used experimentally for treatment of neoplasms and is recommended for Hodgkin's disease and choriocarcinoma. Vincristine, another alkaloid, is used for leukemia in children. Vinblastin is sold as Velban; vincristine, as Oncovin. Crude extracts from different parts of *C. roseus* was tested for antibacterial activity. Extracts from the leaves showed significantly higher efficacy. Study suggests
that bioactive compounds of *Catheranthes roseus* can be a potentially exploited as antibacterial agents. (Mostofa *et al.*, 2007).

Vijai Lakshmi *et al.*, (2013) showed the pattern of inhibition depends on extraction procedure, part of plant used, state of plant, solvent used, and microorganism tested. The ethanolic extract was most active against almost all bacterial organisms tested. Gram positive bacteria were found more sensitive than gram-negative ones. They also isolated two triterpenes and three alkaloids. Two alkaloids, ajmalicine and serpentine showed very potent inhibitory activity against CYP2D6.

Study showed increased wound contraction and tensile strength, increased hydroxyproline content and supports the topical use of *Catheranthes roseus* in wound healing (Nayak and Lexley, 2006). The effects of triadimefon, a triazole compound on the antioxidant potentials and root alkaloid ajmalicine content were studies in two varieties of *C. roseus*, rosea and alba. Triadimefon treatment increased the antioxidant potentials and the indole alkaloid ajmalicine (more in the rosea variety than the alba variety) content. Results suggest triadimefon may be a useful tool for increasing alkaloid production in medicinal plants (Vijai Lakshmi *et al.*, 2013).

*C. roseus* is known to produce a distinct spectrum of terpenoid indole alkaloids. Growth-related decreases in shoot/leaf dat and sgd transcript levels were paralleled by a decrease in shoot/leaf vindoline content. Study of non-colored phenolics in C roseus characterized three caffeoylquinic acids and 15 flavonol glycosides. The scavenging ability of different plant matrices was assessed and a concentration-dependent protective effect was observed for seeds and tissues, with petals found to be most active (Lu *et al.*, 2003).
C. roseus leaves extract made significant changes in each cardiovascular parameter after investigation with hypotensive and hypolipidemic effects in leaves extract treated animals (Pandey et al., 2007). Study of leaves extract of Catheranthes roseus showed potent anthelmintic activity in experimental adult earthworm Pheritima posthuma. There was concentration dependent paralysis and decrease in death time. In the study, the control drug Piperazine citrate showed more potent anthelmintic activity compared to the methanol aqueous, ethanol and ethylacetate extract (Lu et al., 2003).

Study of a dichlormethane: methanol extract of leaves and twigs in a STZ-induced diabetic rat model exhibited hypoglycemic activity. Decreased enzymic activities in liver of diabetic animals were significantly improved after extract treatment. Increased levels of lipid peroxidation indicative of oxidative stress were also normalized by extract treatment (Pandey et al., 2007). Study of extracts of leaves showed it can be used as a prophylactic agent in regions with endemic disease but no in pandemic scale. Leaf yielded indole alkaloids and some phenolic compounds (Prajakta and Jai, 2010).

Vincristine is a dimer-indo-akaloid from the leaves of C. roseus, used in the treatment of acute lymphocytic cell leukemia, Hodgkin's disease and non-Hodgkin disease. Side effects limit its clinical use. Study summarizes its properties, advances in decreasing side effects, and new pharmaceutical approaches (Lu et al., 2003). Study exposed C. roseus to different concentration of heavy metals to observe bioaccumulation efficiency. Total alkaloid was found decreased in the roots of CdCl2 treated plants. Analyses of leaves of treated plant showed 5-10% accumulation of cadmium, but no accumulation of lead at all (Pandey et al., 2007).
Study evaluated the sub-acute oral toxic effects of methanol leaves extract of *C. roseus* on liver and kidney functions in Sprague Dawley rats. Fourteen days of oral administration of 0.1 g/kbw was shown to be safe in female SD rats without any significant damages to the liver and kidney (Kevin *et al.*, 2012).

Study of a plant leaf dichlormethane methanol extract was found to exhibit significant antihyperglycemic effect in alloxan induced diabetic rats. Study evaluated the antioxidant activity of methanolic leaf extracts of *Catharanthus roseus*. Results showed increased activity of SOD and POX antioxidant activities. Study investigated the anticancer and antioxidant activity of *C. roseus*, *Dendrophoe petandra*, *Piper betle* and *Curcuma mangga* aqueous extracts in T47D human ductal breast epithelial tumor cell line. Apoptotic analysis showed *C. roseus* induced apoptosis for 37.67%, compared to doxorubicin for 36.06. Its DPPH radical scavenging activity was 71.87% (Wahyu *et al.*, 2013). It also described the preventive role of *C. roseus* leaf powder in alleviating high-fructose diet-induced insulin resistance and oxidative stress in adult Wistar rats and suggests a potential use as adjuvant for the prevention and/or management of insulin resistance and related disorders. Hypoglycemic activity of extracts from flower, leaf, stems, and roots in normal and alloxan-induced diabetic mice. The aqueous extracts and its alkaloid-free fraction significantly reduced blood glucose in diabetic mice, with a hypoglycemic activity comparable to tolbutamide (Elisa *et al.*, 2012).

Study investigated the hypolipidemic activity of leaf juice of Catharathus roseus in guinea pigs. Results showed significant anti-atherosclerotic activity suggested by reduction in serum lipids and histological findings in the aorta, liver, and kidney. The
result was attributed, possibly, to the antioxidant effect of flavonoid, and probably, vinpocetine-like compound in the leaf juice (Yogesh et al., 2011). Study of a crude aqueous extract of *C. roseus* showed a differential effect on the inhibition of proliferation of Jurkat leukemic T-cells and promoting normal peripheral blood immune cells proliferation (Nor Hazwani Ahmad et al., 2010).

Aqueous flower extract for antidiabetic and antihyperlipidemic potential on alloxan induced diabetes in male albino rats. Results showed significant reduction in blood glucose, reduction in lipid profile, and histological observation of reduced pancreatic fatty changes and inflammatory cell infiltrates (Natarajan et al., 2012) anti-ulcer activity of total extract and fractions of *C. roseus*. Chloroform extracts fractionation and its compounds, vincamine and vindoline, significantly showed protection in the Cold Restraint Ulcer model, confirming its anti-ulcer activity. The effect could be due to its anti-secretory activity (Vijai Lakshmi et al., 2013).

Satish et al, (2009) evaluated 52 plant species including *C. roseus* against different *Aspergillus* species. One of the species tested was *A. fumigatus* but amongst the 12 plants reported to be effective against the said species, *C. roseus* was not reported to be effective against the fungi. This is quite in contrast to the present investigations. Methanolic leaf extract of *C. roseus var. “rosea”* was most effective against *A. fumigatus* as compared to methanolic extract of stem and flower. Whereas methanolic stem extract of *C. roseus var. “alba”* was found to be effective against *A. fumigatus*. Pankaj Goyal et al., (2008) reported antibacterial activity of *C. roseus* against *E. coli* along with 5 other bacterial strains tested. They observed ethanolic extracts were most suitable than methanol and inhibitory pattern amongst the gram
negative bacteria showed maximum inhibition against *Klebsiella pneumoniae* followed by *E.coli*.

Ramya *et al.*, (2008) also evaluated antibacterial activity of crude extracts of *C. roseus*. Ethanol extracts of stem was found to show antibacterial activity. Some inhibition was also reported by ethanol extracts of leaf, root and flower. They found absolutely no activity in methanolic and aqueous extracts of different plant parts of *C. roseus* using disc diffusion assay.

The ethanol extract showed a maximum zone of inhibition (21.15 mm) against *S. typhi* and minimum zone of inhibition (06.24mm) with ethanol extract against *S. aureus*. Further, the methanolic extract observed in maximum (15.61 mm) against *S. typhi* and minimum (05.20 mm) zone of inhibition against *E. coli* (Chinnavenkataraman and Rajendran, 2012).

The leaf extract when tested exhibits, antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Candida albicans* and *Penicillium* species. Among the species tested *Aspergillus flavus* gave better results. Ethanol extract of *Catharanthus roseus* extract showed better result on *Aspergillus flavus* pathogen (Balaabirami and Patharajan, 2012)

Pankaj Goyal *et al.*, (2008) indicated the antibacterial potential of crude extracts of different parts of *Catharanthus roseus*. It is clearly demonstrated that extracts prepared from dried parts revealed better antibacterial activity than those extracts
prepared from fresh plant part. Almost all parts of the plant showed antibacterial potential in dried form while only leaf and root extracts showed zone(s) of inhibition in their fresh states.

Crude ethanolic fractions of *Catharanthus roseus* were tested against all the isolates. *Pseudomonas aeruginosa* (29mm), were highly sensitive to the ethanol fraction followed by *Staphylococcus aureus* (25mm), *Escherichia coli* (24mm), *Klebsiella pneumoniae* (18mm) and *Streptococcus pyogens* (15mm). In case of Crude methanolic extract *Staphylococcus aureus* (25mm), *Klebsiella pneumoniae* (24mm), *Escherichia coli* (21mm), *Pseudomonas aeruginosa* (20mm), *Streptococcus pyogens* (16mm) also shows their sensitivity. Soxhlet methanolic extract shows highest sensitivity against *Staphylococcus aureus* (16mm) followed by *Escherichia coli* (13mm), *Klebsiella pneumoniae* and *Streptococcus pyogens* (12mm) *Pseudomonas aeruginosa* (9mm). Under Soxhlet ethanolic extraction sensitivity was recorded in *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Streptococcus pyogens* as 22mm, 21mm, 18mm, 15mm and 12mm respectively (Sheeraz et al., 2013).

The antimicrobial activity from ethanol leaf extract of *Catharanthus roseus from Saudi Arabia* was investigated against some human pathogenic microorganisms (*Staphylococcus aureus* and *E.coli*) as well as pathogenic fungi (*Candida albicans*). The tested extracts showed very strong antimicrobial activity against all organisms. The antimicrobial activity was evaluated by measuring the zone of inhibition using disc
diffusion method. The strongest inhibition activity of the leaf extract was observed against *Staphylococcus aureus* (15 mm zone) at 100 mg/ml of leaf extract followed by *E.coli* which showed 11 mm inhibition zone at 100 mg/ml leaf extract. Ethanol leaf extract also demonstrates antifungal activity against the pathogenic fungi *Candida albicans* (12 mm zone of inhibition) (Amjad, 2012).

*Catharanthus roseus* is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities (Jaleel *et al.*, 2008). Antibacterial potential in crude extracts of different parts of *C. roseus* against clinically significant bacterial strains has been reported (Muhammad *et al.*, 2009). In the last few decades bacterial resistance to antibiotics has become a serious therapeutic problem and the rate at which new antibiotics are being produced is slowing, (Russell, 2002). Thus, the search for novel antimicrobial agents is of the utmost importance (Gootz *et al.*, 1990). Worldwide attention has been shifted towards finding new herbal chemicals for the development of new drugs. These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery (Nisbet and Moore, 1997).

Satish *et al.*, (1999) illustrated that a Gram-positive bacteria were more susceptible to this extract as compared to Gram-negative bacteria species. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms (Pankaj *et al.*, 2008). Results of this work showed that the extraction of antimicrobial substances by organic solvents is better as compared to aqueous extracts (Thongson *et al.*, 2004). The polarity of antibacterial compounds
make them more readily extracted by organic solvents, and using organic solvents does not negatively affect their bioactivity against pathogenic bacteria species. Despite what many researchers have reported that *Candida albicans* are very resistant fungi, this work demonstrate that ethanol leaf extract of *Catharanthus roseus* is effective against this pathogenic fungi. This result is in agreement with other studies showed that the leaf extract of *Catharanthus roseus* is very effective on *Candida albicans* (Uniyal et al., 2006).

Alkaloids of *C. roseus* are well known for their hypoglycemic, sedative and antihyperglycemic properties and used in anticancer therapy (Chtatopadhyay et al., 1991). Even at a minimal concentration of 10 μg and 20 μg the extracts of *C. roseus* have shown a good zone of inhibition. *Shigella sonei* showed sensitivity by forming a zone of 15 mm in diameter at a concentration of 20μg/mL and formed a zone of 10mm at a concentration of 10 μg/mL. *Proteus vulgaris* at a concentration of 10 μg/mL formed a zone of 8mm in diameter and at a concentration of 20μg/mL formed a zone of 16mm in diameter. *Klebsiella pneumoniae* at 10μg/mL formed a zone of 13mm in diameter and at 20μg/mL formed a zone of 14mm in diameter. *Staphylococcus aureus* at 10μg/mL formed a zone of 9mm in diameter and 20μg/mL formed a zone of 16mm in diameter. The trace elements in the *C. roseus* root extract may be directly or indirectly helpful in the management of many diseases. These compounds may play a major role in health care and disease control of human body, there is ample scope for their exploitations in further investigations (Vijayalakshmidevi et al., 2011).
The antibiogram of 10 indole alkaloids and 4 semi synthetic variables obtained from three plants, *C. roseus*, *Vallesia antillana* Wood and *Ervatamia coronaria* Staph, of the family Apocynaceae growing in Cuba was assessed in vitro. The alkaloids and the variables used were catharantine, vindoline, vindolinine, perivine, reserpine, tabernaemontanine, tetrahydroalstonine, aparicine, vindolinic acid, reserpic acid and vindolininol. These were faced to 40 bacterial strains from the genera *Salmonella*, *Shigella*, *Proteus*, *Escherichia*, *Pseudomonas*, *Staphylococcus* and *Corynebacterium* as well as to fungi and yeasts from the genera *Aspergillus*, *Cunnighamella*, *Candida* and *Saccharomyces*. The method involving cylindric sections in a double agar layer was applied and after 24-48 h of incubation at 25°C for fungi and yeasts and 37 °C for bacteria, inhibition zones are reported in millimeters. *C. roseus* shows various aspects of studies. Experiments were conducted on the antibiogram of *C. roseus* on *B. cereus* and *B. megaterium* and it was found that among various extraction procedures with different solvents, crude ethyl acetate extract was very effective antimicrobial whereas the same extract from the plants grown in sandy soil did not show any antibiogram. From these observations, it is clear that soil conditions play a major role in determining phytochemical constituents. Plant extracts (leaf, stem, root and flower) of two varieties of *Catharanthus* using methanol, ethyl acetate and acetone solvents for their in vitro antibiogram against *Staphylococcus aureus*, *Streptococcus* sp., *Bacillus subtilis* and *Klebsiella* sp. (Sathiya et al., 2008).

**Traditional uses**

The species has long been cultivated for herbal medicine and as ornamental plant. In Ayurvedha (Indian traditional medicine) the extracts of its roots and shoot though poisonous, is used against several disease. Over to different alkaloids, including
vincristine and vinblastine, have been extract from *V. rosea* The active ingredients known as tannins, which are used for making medicines to treat a number of diseases. In traditional chinese medicine extracts from it have been used against numerous diseases, including dibetes, malaria and Hodgkin’s lymphoma. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia and Hodgkin’s lymphoma. Extracts from the dried or wet flowers and leaves of plants are applied as a paste on wounds in some rural communities. The fresh juice from the flowers of *C. roseus* made into a tea has been used by Ayurvedic physicians in India, for external use to treat skin problems, dermatitis, eczema and acne. Daily supplements made with the active ingredients found in *vinca rosea* help to improve blood supply to the brain and increase the level of oxygen and glucose that the brain can effectively utilize. The results of maximum antibacterial activity were identified with Ethanolic and Methanolic (leaf and flower) extract of *Catharanthus roseus*. *Pseudomonas aeruginosa* and *Staphylococcus aureus* shows more antimicrobial activity of the ethanolic extract might be due to the presence of unique phytochemical constituents (Komathi and Vanmathiselvi, 2014).

Antimicrobial activity of ethanolic, methanolic, aqueous and chloroform extracts of leaves, stems and flowers of *Catharanthus roseus* were studied against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger* and *Aspergillus fumigatus* by agar well diffusion method. Ethanolic leaf extract of *Catharanthus roseus* had shown antimicrobial activity against *Candida albican*, *Pseudomonas aeruginosa*, and *Aspergillus niger* (with zone of inhibition 14, 13 and 8 mm respectively). Ethanolic stem and flower extract had shown antimicrobial
activity against *Staphylococcus aureus* and *Aspergillus niger* (with zone of inhibition 19, 6 mm by stem extract and 8, 10 mm by flower extract). Maximum antifungal activity against *Candida albicans* was exhibited by methanolic flower extract of *Catharanthus roseus* (18 mm zone of inhibition) followed by methanolic leaf extract (13 mm zone of inhibition) and then methanolic stem extract (11 mm zone of inhibition). Chloroform flower extract of *Catharanthus roseus* had shown antibacterial activity against gram negative bacteria *Pseudomonas aeruginosa* (with zone of inhibition 4 mm). Aqueous leaves stem and flower extract of *Catharanthus roseus* were not effective against any bacterial and fungal strains. Hence the ethanolic extract is more useful for preparing the antibacterial drugs while metanolic extracts are useful for antifungal extract (Sonia et al., 2013).