Urinary tract infection is one among the commonest infectious inflammatory disease (Abolfazl Mahyar et al., 2014) of the human. It is one of the second most common infections in human throughout the world. There are estimated at 150 million urinary tract infections per year worldwide. It is among the commonest bacterial infections responsible for more than 8 billion hospital visits in India (Rudramurthy et al., 2015). UTI is one of the most common health problems in India (Poongothai et al., 2014). It affects all age groups across the lifespan (Manal, 2015). Though various factors associated with the incidence of UTI, bacteria play a major role (Acharya et al., 2011). Proper sample collection is necessary for the complete recovery of uropathogens from the urinary tract. In India, mid stream urine is used for the isolation of bacterial pathogens of the urinary tract. It is a simple and easy method and represents actual infectious status of an individual. In this study 498 mid stream urine samples were collected across different age groups and both sexes. A supportive evidence of mid stream urine sample collection for the study of uropathogens was quoted by Acharya et al., (2011) from Nepal. Likewise Mwaka et al., (2011) also stated that clean caught mid stream urine was best suited for complete recovery of bacterial pathogens.

Out of 498 urine samples, 64% samples were collected from the female cases (n=321) and 36% (n=177) from male. Higher incidence of the female may be due to the short urethra; resulting in easy accessibility of bacteria from anus to urinary tract (Rajan, 2011). Similar studies carried out at various parts of the world adds this
evidence (Mwaka et al., 2011and Soto et al., 2011). Ismaili et al., (2011) from Belgium indicated that 63% UTI cases were from females and 37% were males. Khan and Zaman, (2006) reported that 83% of higher incidence of UTI was noted among female. Salo et al.,(2011) from Spain also indicated similar type of incidence of UTI among female (Figure 1).

One of unpublished data from India visualized that short urethra of female and nonhygenic condition among the rural population and urban slum population may be the reason for the higher incidence of UTI among female in developing countries like India. Higher incidence of UTI among the age group of 61-80 years was noticed in this study. Improper management of elders and poverty among Indian elders and less immune power could be a cause for the higher incidence of 61-80 years in adult. Shakya et al., (2014) also reported that significant bacteriuria was detected in 34% of the total subjects, mostly from patients with Chronic Kidney Disease. The higher incidence of bacteriuria in female (40.40%) than in male (27.52%) mostly occurred in elderly patients.

As already known age plays a prime role in the incidence of infectious diseases. Like incidence and percentage of urinary tract infection appeared to be more prevalent among the age group of 61-80 constituting 41% out of the total four age groups considered. Likewise the lower percentage of occurrence was among the age group of 41-60 perhaps with the lowest percentage of 12 %. Though to prove supportive evidences for our studies carried out by Shakya et al.,(2015), who stated about the
higher female UTI incidences in all the age groups. Similar reports were done by Sharma et al., (2011). But Chukwu et al., (2011) showed lower incidence of UTI among the age group of 1-10 in contrast.

Urinary tract infections are due to inflammatory reactions. Microorganisms trigger inflammatory reactions in urethra and kidney, which leads to painful urination, purulent discharge etc., along with fever. These common symptoms associated with urinary tract are considered as UTI. During this condition urine is passed with bacteria, pus cells and inflammatory cells. Microscopic examinations of urine sample revealed the presence of bacteria in all the samples dealt hence proving the bacteruria (Figure 4). In the work undertook bacteria along with pus and inflammatory cells strongly correlate UTI among different age groups. Mbanga et al., (2010) expressed that bacteruria is the main indication of UTI. Similarly out of 2941 urine samples collected, 547 samples (18.5%) yielded significant bacteriuria (Niranjan and Malini, 2014).

Urinary tract infections are common conditions worldwide and the pattern of antimicrobial resistance varies in different regions. It is one of the commonest domiciliary and nosocomial bacterial infections, comprising of a variety of clinical conditions caused by microbial invasion of tissue lining the urinary tract which extends from the renal cortex to urethral meatus. Infection of adjacent structures such as prostrate and epididymis is also included in this entity. It also refers to the presence of bacteria undergoing multiplication in urine within the urinary drainage system (Hooton, 2000; Warren et al., 1999). The relationship between sex and isolated bacterial agents of UTIs is elaborated in this study. Prevalence of different pathogens is dependent on several population attributes, sample size and hygienic conditions of the patients. E.
coli has been proved to play the major role in urinary tract infection in this work undertaken.

Identification of bacteria is one of the arts of microbiologists. Though molecular methods suggest the nature of bacteria, it should be correlated with basic cytology and physiological features. A number of chromogenic and non chromogenic media are available along with a plenty of biochemical tests recently. These tests were applied for the identification of bacteria based on its basic metabolic activities. Selective cum differential media and chromogenic media were used for the identification of bacteria. Each organism had its own metabolic process thereby indicating colour and consistency on these media (Koneman et al., 1994). They also gave selective and differential features of *Escherichia coli*, *Enterococcus sp.*, *Staphylococcus aureus*, *Pseudomonas sp.*, *Klebsiella sp.*, and *Proteues sp*. For example *Escherichai coli* produce metallic sheen colonies, which are due to mixed acid production from glucose. Various growth patterns on culture media and biochemical features (Table 5.1-5.7) were expressed by uropathogens isolated from the reported cases of UTI in our studies. The use of culture method and biochemical features are a notable feature in the process of bacterial indentification was justified by Daoud and Afif (2011).

Six different uropathogens were recognized out of the multiple urine samples collected in the work undertook and E. coli justified its dominance with 51% incidence (Figure 5). Similar pattern of of uropathogenic isolate was carried out at Pakistan by Bashir et al. (2008) whose results revealed the presence of *Escherichia coli, Enterococcus sp.*, *Staphylococcus aureus*, *Klebsiella sp.*, and *Proteues s.* and the absence of *Pseudomonas*. Variation in the percentage of recovery between the two countries i.e., India and Pakistan was indicated. Five percentage recovery of
Enterococcus was reported in studies carried out in our neighbor country, which three times lower than the present studies (17%). But a common interpretation that Escherichia coli were prevalent pathogen in adult was laid.

Recovery rate of E. coli was higher in both the sexes when compared to other uropathogens (Table 5.8). Similar reports were published by the work carried out by Bashir et al., (2008).

Bacterial pathogens belonging to the enterobacteriaceae family are more prominent in causing urinary tract associated infection among Indian population. Perhaps the result of our work undertook proved the same with a 70% of uropathogens belonging to the enterobacteriaceae family of the total isolates (Figure 7). Justifying the same similar but increased percentage recovery of enterobacteriaceae incidence was noted by Yamamichi et al., (2012) and Mbanga et al., (2010) proving that the enterobacteriaceae are the most frequent pathogen detected, causing 84.3% urinary tract infections. Daoud and Afif (2011) isolated Klebsiella pneumonia, Pseudomonas aeruginosa, Enterococcus sp., S. agalactiae from a urine sample. Our report was inlinded with the report given by Yamamichi et al., (2012). Mbanga et al., (2010) expressed from Zimbabwe that 40.3% UTI pathogens were E. coli followed by 16.1% Coagulase negative Staphylococci, 11.2% Klebsiella sp, 8% Staphylococcus aureus, 8% Group A Streptococci and 8% were Klebsiella oxytoca.

Nadia Gul et al., (2004) reported that the frequency of gram negative enteric bacteria causing UTI is more than gram positives with an isolation rate of 47.6% E. coli, 9.2% Pseudomonas, 7.6% Klebsiella, 6% Enterobacter, 4.6% each of Proteus, Serratia, Staphylococcus, Streptococcus, Enterococcus and 3% Bacillus. Similar kind of frequency was observed in our studies too (Figure 6) except for the absence of
Bacillus, Serratia, Enterobacter and Streptococcus. Likewise frequency of isolation was also varying with the previous reports. India like tropical countries showed higher incidences of *E. coli* and *Enterococcus* because they are considered as a faecal bacteria contaminants of water pool and easily invades susceptible populations.

The occurrence of *Klebsiella* (15.9%) and *Pseudomonas* (11%) with *E. coli* (67.5%) accounting for the major part was reported by the work carried out by Mahesh et al., (2010) from south India. Recovery of *Klebsiella* and *Pseudomonas* was also noticed in our work but of a meager incidence.

In the present study, *Escherichia coli* (51%) topped the list of organisms causing UTI and proved itself as a major causative agent of UTI. The same was reported by by different workers from various parts of the world. Arslan et al., (2005) stated that *E. coli* was a causative agent in 78% UTI. Similarly Chen et al., (1998) in Taiwan reported *E. coli* as a common pathogen. Peterson et al., (2006) from USA reported the same percentage of *E. coli* incidence. Nicolle (1997) expressed that *E. coli* as a common pathogen of UTI with a worldwide prevalence rate of 21-54%. This frequency of *E. coli* isolation is also supported by Gales et al., (2002). Daoud and Afif (2011) also reported that *E. coli* was the most frequent isolate throughout 10 years accounting for 60.64% of incidence in Lebanon. Interestingly a different version of *E. coli* recovery rate was indicated by Matsuo et al., (2012). They isolated only 19.4% *E. coli* from ovarian cancer patients UTI infection. Infection in cancer patients may be influence by different pathophysiological condition.
This undertaken study revealed *E. coli* as a major predominant pathogen of UTI. This was also backed by Siedelman *et al.*, (2012), Walters *et al.*, (2012), Yamamichi *et al.*, (2012), Acarya *et al.*, (2011), Sharma *et al.*, (2011), Chlabicz *et al.*, (2011) and different authors from different countries. But there is no proper report from India especially from Tamilnadu. Virulent and molecular profile studies are needed for completing the nature of *E. coli* from UTI.

About 127 pure *E. coli* isolates were subjected to antibiotic sensitivity assay. The present evaluation brought out today’s microbial sensitivity pattern against ten different antibiotics that are commonly used and recorded an alarming situation of antibiotic resistance. All the isolates were considered as multiple drug resistant uropathogenic *E. coli*. Only Cephalosporin was found to be comparatively effective (35% sensitive). About 97% organisms were resistant to Cephadoxime (Table 5.9). Siedelman *et al.*, (2012) and Walters *et al.*, (2012) reported that 76.5% of community acquired UT infections were due to *E. coli*. Among them, 60.6% of *E. coli* were ESBL producers i.e.. Multidrug resistant isolates. They again stated that the resistance rates of *E.coli* detected from urine culture was found to be 100% for Cefpodoxime (CF), 93% Novobiocin (NV), 89% Vancomycin (Va), 86%, Ampicillin (A) respectively. Additionally, the most sensitivity rates were reported for Imipenem (90%) followed for gentamycin (33.3%) (G).

Bonkat *et al.*, (2013) expressed that the rate of ESBL-EC positive urine samples an increased significantly during the study period (3 in 2001 compared to 55 in 2010). Our report also revealed similar rise in the incidence of ESBL producers, that is 6/11 (55%) strains were found to be ESBL producers. According to them the most active
agents were Imipenem, Meropenem and Fosfomycin (100%), followed by Amikacin (99.1%) and Nitrofurantoin (84%). The least active substances were Ampicillin-clavulanate (20%), sulfamethoxazole (28%) and ciprofloxacin (29.6%). The rate of urinary ESBL-EC isolates is increasing. Their susceptibility to nitrofurantoin, fosfomycin, and carbapenems is excellent, whereas Ampicillin-Clavulanate, Sulfamethoxazole, and Ciprofloxacin to demonstrate only low susceptibility. In particular, the use of ciprofloxacin should be strictly avoided in urologic patients with suspicion for an ESBL-EC urinary tract infection as well as routine antibiotic prophylaxis prior to urological interventions if not explicitly indicated by current international guidelines or local resistance patterns.

Drug resistance in microbes becomes a big problem along with the emergence of new infectious diseases. Microbes acquire resistance against the available antibiotics by the production of drug degrading enzymes, harbouring resistant plasmids, alteration of metabolic pathway, etc., (Down, 1999 and Michael et al., 2003).

Sabir et al.,(2014) reported that UTI caused by antibiotic resistant bacteria have marginally raised in recent times. Similar kind of report was presented in our study. Eleven strains were selected from 127 test strains based on its resistance pattern (Table 5.11), out of which nine strains found to possess biofilm producing ability. All the strains were resistant to ceftriaxone. UPEC exhibits multiple numbers of virulence factors. It facilitates colonization of \textit{E. coli} in the bladder (Hilbert \textit{et al.}, 2012). They reported that, Twenty-seven (35.1 %) and 50 (64.9 %) ESBL-producing UPEC strains were isolated in neonates and infants, respectively. Of 70 strains investigated for the presence of virulence factors, adhesions were detected in 48.6% strains (8.6% in the
neonate and 40% in the infants group) giving a statistically significant difference in adhesion expression between the two groups. Bedenić et al., (2012) reported that 84.3% of uropathogenic E. coli strains produce haemolysin producing strains. Similar, with a little variation was expressed in our study. Various conditions of the environment play a vital role in expression of the gene product. Among 11, 6 isolates showed ESBL and 9 organisms showed biofilm producing ability (Table 5.12).

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 of uro-epithelium, promoting colonization and persisting in the urinary tract, inducing a local inflammatory response and promote tissue lesions (Mulvey, 2002). Thought our results were in line with the report given by Markoviae et al.,(2013), who stated that almost 60% of isolates produced two or three virulence factors; only 3.8% produced none of the virulence factors. Our study showed a little deviation where all the tested urinary isolates had any one of the virulence factors which was expressed when assessing biofilm production and betalactamse production (Table 5.12).

RAPD patterns of most resistant E. coli uroisolates were carried out using standard random primers. The genetic diversity among E. coli species is determined by the PCR amplification method. In this study, ten random primers were used to determine the genetic diversity among E.coli by PCR amplification (Table 5.13 and 5.14).
The primers were subjected to optimize conditions for RAPD PCR. This primer exhibited different band patterns among the *E.coli*. The amplified fragments ranging from 100 bp above 1100bp (Plate I and II). RAPD is a simple and widely used method for strain differentiation, since it does not require any specific knowledge of the DNA sequences in the target organism. Though RAPD technique has certain limitations, still it is being used as a molecular typing method due to its simplicity, sensitivity, flexibility and relatively low cost (Abou-dobara *et al.*, 2010; Intrakamhaeng and Komutarin, 2012). Haryani *et al* (2008) found that 4 RAPD profiles among seven studied *Enterobacter cloacae* whereas Trautmann *et al* (2006) showed isolates of *P. aeruginosa* had similar fingerprints which indicated that a similar distribution of genotypes. This study clearly indicated that the place of survival and community setup also responsible for the transfer of infectious agents. None of the strains were showed 100% similar RAPD pattern (Figure 11).

In the present study, 11 isolates were utilized for the antibiotic resistance gene analysis by multiplex PCR. CTX-M, TEM, SHV and OXA gene primers were used for antibiotic resistance analysis. Our isolates E8 showed three antibiotic resistance gene. CTX-M gene was found in 5 strains, TEM was found in 4 strains. None of the strains possess OXA gene (Table 5.15 and Figure 13). Bedenic *et al.*, (2012) reported that TEM gene was detected in 28 % of isolates, SHV gene in 74 % and CTX-M gene was detected in only 2.5% isolates. TEM and SHV were simultaneously detected in 25% of isolates. They also concluded that infection control measures should be employed and
the consumption of expanded-spectrum cephalosporins in the hospital should be restricted.

The presence of CTX-M type extended spectrum of beta lactamases was reported from different parts of the world. CTX – M was a major reason for antibiotic resistance were reported by Lepeule et al., (2012) and Randall et al., (2011) from England, Titelman et al., (2011) from Sweden, Bourjilat et al., (2011) from Morocco, Narciso et al., (2011) from Portugal, Chouchani et al., (2011) from Tunisia and Akram et al., (2011) from India.

The study expressed that all the isolates belong to ESBL strain. This increases the complications of infection treatment. Siedelman et al., (2012) revealed that 60.6% of the health facility acquired and 76.5% of the community acquired ESBL infections were due to E. coli (Figure 13). All the strains were found to possess the plasmid (Figure 12).

Plasmid is one of the most important known mediators in facilitating the fast spreading of antibiotic resistance among bacteria (Dale and Park, 2004). Plasmid isolation study revealed that all MDR isolates harboured two plasmids (Figure 12). Our result is in agreement with the findings of Shahid et al (2003) and Oppegaard et al (2001), as they have isolated single plasmid of 48.5 kb and 65 kb in MDR isolates of Pseudomonas aeruginosa and lactose-fermenting Coliform, respectively. Bourjilat et
al., (2011) isolated seven ESBL producing *E. coli* from 535 *E. coli* isolates. Most of them expressed CTX-M, TEM and SHV gene.

PCR was used to detect the presence of virulence genes which play an important role in pathogenicity of *E. coli* by using specific primer. Intimin protein is responsible for the intimate adherence between bacteria and the enterocyte membrane. Categories of *E. coli* that differs in their virulence factors contain *eae* gene encoding for intimin as part of pathogenicity island EPEC and EHEC (Rawa’a Al-Chalabi *et al.*, 2010). Intimin, encoded by *eaeA* gene, is responsible for adherence of UPEC. Our data revealed that all *E. coli* isolate E8, regardless of their source, harboured the gene (Figure 14). These observations suggest that the *eaeA* gene may play a major role in the pathogenesis of UPEC, maybe by facilitating adherence to uroepithelial cells. It also seems to contribute to the ability of UPEC strains to cause bacteremia (Matar *et al.*, 2005).

In the present study, plasmid is cured using elevated temperature (45°C), which results in loss of the plasmid. Fortina and Silva, (1996) obtained curing of 14.3 kb plasmid in *Lactobacillus helveticus* strain ILC 54 at 45°C. The plasmid cured cells became sensitive to all previously resistant antibiotics, which revealed that antibiotic resistance marker genes were located in plasmid (Abhay, 2012). It is clear from Elias *et al.*, (2013) that the elevated temperature has a remarkable effect on all antibiotic resistance conferred by the bacterial isolates. They stated that all isolates lose their resistance to all antibiotics used in a range of 66-100%, 61-100%, 59-98%, 62-100%,
55-100% and 67-100% in P1, P6, P8, P10, P14 and P21 respectively. From the obtained results, it can be concluded that curing by elevated temperature is an efficient method compared to others. This may be due to the fact that the enzymes of DNA replication become more affected by this temperature (Table 5.16 and 5.17). Our interpretation involves changing the shape (folding of the polypeptide) of the enzyme responsible for DNA replication of plasmids, which results in the inactivation of enzymes. It was observed that inactivation of these enzymes may be due to the change in the folding of polypeptide at this temperature, that is, enzymes are sensitive to elevated temperature (Li et al., 1994). Studies have been made on the effect of elevated temperature on DNA synthesis and plasmid curing. Bennett (2008) reported that there is a clear effect of elevated temperature on curing the plasmid DNA content from isolates of P. aeruginosa which is in agreement with the results of this study. As ESBL-plasmid-curing of the strains applied in this study did not work treating bacteria with ethidium bromide or acridine orange. This method was adequate to construct viable PCVs and our knowledge we are the first ones to describe a successful “curing” of ESBL-plasmids, which carry genes for toxin-antitoxin systems. Our study revealed that after curing none of the organism showed plasmid, which are evident in plasmid isolation study (Figure 15).

Medicinal plants are selected for the screening of bioactivity based on their traditional use (Morton, 1987). Phyllanthus emblica fruit, Catharanthus roseus leaves, Aegle marmelos fruit and Mangifera indica seed kernel were selected for the screening of bioactivity based on traditional use (Morton, 1987). Today people turned towards TSM due to ill effects created by the modern medicine and antibiotic resistance among
Siedelman et al., (2012) and Walters et al., (2012) reported that 76.5% of the community acquired infections are due to multidrug resistant microorganisms. They reported that pathogens like E. coli, Salmonella, Vibrio and Staphylococcus aureus were 100% resistance to ciprofloxacin, cephalosporin, cefodoxime and are 98% resistant to erythromycin and bacitracin and 93% to novobiocin and tetracycline. Most of the pathogens showed multiple virulent factors (Tchesnokova et al., 2011). Medicinal plants are considered as the best alternative source of medicine and used to overcome antibiotic resistance and highly virulence of the pathogens.

In the present study, four plant materials were selected for screening antibacterial activity against UPEC strains. All the plant materials showed effective antibacterial activity. Among the plants Phyllanthus emblica fruit showed best activity and Aegle marmelos fruit showed least activity (Table 5.16 to 5.18). Lower activity of Aegle marmelos may be due to lower concentration of active principle. Effective activity of this plant was evidenced by Sujatha and Rajan (2014). Mohan et al., (2005) reported that the aqueous, acetone and petroleum ether extract of A. marmelos were found to be effective against B. coagulans, B. subtilis, B. thuringiensis, P. aeruginosa and S. aureus. They analysed the antibacterial activity only by making use of gram positive bacteria. The present findings support the applicability of A. marmelos in traditional system for its claimed uses and can be recommended by the scientific community as an accessible alternative to synthetic antibiotics.
Antibacterial activity of the study also supported by Mohan et al., (2005). They reported that the aqueous, acetone and petroleum ether extract of A. marmelos were found to be effective against B. coagulans, B. subtilis, B. thuringiensis, P.aeruginosa and S. aureus. They analysed the antibacterial activity only by making use of gram positive bacteria. The present findings support the applicability of A. marmelos in traditional system for its claimed uses and can be recommended by the scientific community as an accessible alternative to synthetic antibiotics.

The present study exhibited good antimicrobial activity, which could be due to the phytochemicals present in the extracts. One of the previous studies indicated the presence of steroids, terpenoids, flavonoids and phenolic compounds in aqueous and alcoholic extracts (Rajan et al., 2011). This report also indicated that tannins were only present in alcoholic extracts along with other reported chemicals (Table 5.19). This is also evidenced by Rajeshwari and Ramachandramurty (2013). Tannins are known to be useful in the treatment of inflammed or ulcerated tissues and they have remarkable activity in cancer prevention and are thought to be responsible for coagulating the wall proteins of pathogenic organisms. Thus, A. marmelos alcoholic extract containing this compound may serve as a potential source of bioactive compounds in the treatment of infectious diseases. Flavonoids have been shown to exhibit their actions through effects on membrane permeability and by inhibition of membrane bound enzymes such as the ATPase and phospholipase (Li et al., 2003). They also serve as health promoting compounds as a result of their anion radicals (Hausteen, 1983).
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 seed, containing 15% tannin, served as astringents in cases of diarrhea, chronic dysentery, catarrh of the bladder and chronic urethritis resulting from gonorrhea. 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 seem to be a safe source of antioxidant (Rukmini and Vijayaraghavan, 1984).

This result suggests that all extracts possess compounds with antimicrobial properties and can be used as antimicrobial agents that can be used as therapy for infectious diseases in human. Extracts had an inhibition zone diameter between 10mm to 16mm, which was higher than the standard antibiotic, hence it is suggested their effectiveness as antimicrobials. The active components in the crude extract may be acting synergistically to produce antimicrobial effects (Elloff, 1998).

The antimicrobial activity A. marmelos has been traditionally used for the treatment of various infectious diseases and scientific studies also 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 ciproflaxacin 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).

*Mangifera indica* seed kernel showed variable antimicrobial activity. Sairam et al. (2003) reported that *Streptococcus sp.* and *Proteus vulgaris* were inhibited by methanolic and aqueous extracts of *Mangifera indica* seed kernel. *Mangifera indica* was found to have significant antibacterial activity (Sairam et al., 2003). *Mangifera indica* seed kernal is a promising source of food additive and enhance oxidative stability of food and used as food preservator (Toshihide et al., 2000). Ethanol extracts of *Mangifera indica* seed kernel was active against eighteen species of food borne pathogens (Toshihide et al., 2000). The seed of *Mangifera indica* is reported in traditional medicine to cure vomiting, dysentery and burning. Decoction of seed kernel is generally prescribed for diarrhoea (Kirthikar and Basu, 1984). *Mangifera indica* has been used along with other classical traditional antidiarrhoal drugs like *Holarrhena antidysenterica* and *Oxoylum indicum* (Singh and Chathurvedi, 1981). Das et al., (1989) reported the antibacterial activity of *Mangifera indica* seed kernel against gram negative pathogens. These studies establishes the use of *Mangifera indica* as an antidiarrhoal medicine as claimed by the traditional medicine.

Most of the identified components with antimicrobial activity extracted from plants are aromatic or saturated organic compounds which are more soluble in polar solvents such as water and organic solvents. However water extracts were less potent.
This can be attributed to the presence of water-soluble compounds such as polysaccharides and polypeptides, which are commonly more effective as inhibitors of pathogen adsorption and have no real impact as antimicrobial agents (Ncube et al. 2008). The antibacterial activity demonstrated by water extract provides the scientific bases for the use of water extracts in traditional treatment of diseases. There are also reports in literature that organic solvent is a better solvent for consistent extraction of antimicrobial substances for medicinal plants (Elloff, 1998). This may be attributed to two reason, firstly, the nature and potentiality of biologically active components (alkaloids, steroids, flavonoids, essential oils, biterpenoids), which could be enhanced in the presence of methanol. Secondly, the stronger extraction capacity of methanol could have produced greater number or amount of active constituents responsible for antibacterial activity (Jeyachandran et al., 2010). This is also proved in this study in which ethanolic extracts exhibited the highest antibacterial activity against all clinical isolates tested.

There are many reports available that plants have been evaluated in vitro for their antibacterial potency against some important human pathogenic bacteria (Kulkarni, 1999; Hiremath et al., (1993); Srivastava and Lal, (1997); Adelakun et al., (2001); Verma and Dohroo, (2003); Singh and Singh, (2005)). Gram positive bacteria were more susceptible than that of gram negative bacteria in response to the fruit pulp extract observed in the present study. It is in line with the previous reports (Patni et al., 2005; Karou et al., 2005). Scherrer and Gerhardt (1971) reported that gram positive bacteria have outer peptidoglycan layer which is not an efficient barrier. The gram negative bacteria have an outer phospholipidic membrane that makes the cell wall
impermeable to lipophilic solutes, while the prune constitute a selective barrier to hydrophilic solutes with an exclusion limits of about 600 Da. Many results confirmed these observations that most plant extracts were found to be more active against gram positive bacteria than gram negative ones (Nikaido and Vaara, 1985; Kelmanson et al., 2000). Similar type of results were reported by Masika and Afolayan, (2002) and Rahman and Moon, (2007).

Antimicrobial property of a plant depends on its biologically active phytoconstituents. A wide range of antiinfective actions have been assigned to tannins (Haslam, 1996). Some authors have found that more highly oxidized phenols are inhibitor. Flavonoids are synthesized by plants in response to microbial infection. Terpenoids are active against bacteria (Ahmed et al., 1993), fungi (Ayafor et al., 1994), viruses (Fujioka and Kashiwada, 1994) and protozoa (Ghoshal et al., 1996). Hence, the plant which was subjected to this investigation reveals the presence of active phytochemicals, which exhibits many beneficial properties. Aegle marmelos fruit extracts inhibited the growth of all types of microbial population (Table 5.17). Tannins and coumarins found in the Aegle marmelos fruit precipitates cell wall proteins of microorganisms and also suppress prokaryotic DNA replication.

The fruits of Emblica officinalis 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). Phyllembin, isolated from the ethanolic extract of the fruit pulp has been found to potentiate the action of adrenaline in vitro and in vivo. Active principles of this plant are Tannins and Gallic acid. The fruit is a very rich source of vitamin C (Ghosal et al., 1996). The fruit ethanol extract demonstrated anti H. pylori activity In Vitro (Mehrotra et al., 2011). 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. 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.

*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 as 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.

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 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). The ethanolic extract showed a maximum zone of inhibition (21.15 mm) against S. typhi and minimum zone of inhibition (06.24mm) with ethanolic 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 leaves extract were tested exhibited the antifungal activity against Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Candida albicans and Penicillium species. Among the species tested Aspergillus flavus gave better results. Ethanolic extract of Catharanthus roseus extract showed better result on Aspergillus flavus pathogen (Balaabirami and Patharajan, 2012). 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) (Sheeraz et al., 2013).

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, leporsy, myalgia, smallpox, spermatorrhoea, leucoderma, eye disorders, ulcers, mental illnesses, nausea, sores, swelling, thirst, thyroid disorders, tumors, ulcers and upper respiratory tract infections (Ashkenazi and Pickering, 1989; Daswani et al., 2009). It is usually used for household consumption and traditional
medicine, but some are planted for trade in particular regions (Subhadrabandhu, 2001). 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).

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. 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 gastrointestinal 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, antidyssmenorrheic, anti scorbatic, 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. Seed kernels are used to cure chronic diarrhoea, to expell tape worms and other worms in ulcers (Warrier, 1994 and Kurian, 2001). 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 (Sharma et al., 1971). 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 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, mangos are used for strengthening nervous and blood systems, removing toxins from blood and treating anaemia. (Nadkarni, 1987; Warrier, 1994; Anonymous, 1962).

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 gonorrhea. 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), diarrhea (Ponce et al., 1994).

In this present research study, molecular modeling of ligands and its target was focused on how the alkaloid compounds from Phyllanthus emblica (Phyllantidine and Phyllanthine) (Bharambe et al., 2013). These ligands were subjected for bind to Eae iuintimin (E. coli) protein structurally using Insilico tools and database. Insilico drug designing has been used to find out tailor made drugs to bind to the conserved regions in the virulence factor proteins present in the pathogenic bacteria (Sundar and Nelson, 2006). The Phyllanthine, Phyllantidine and gentamicine potentially inhibited the target protein Eae intimin (E. coli). Based on the molecular drug docking and binding affinities of the target protein Eae intimin (E. coli) with the two alkaloids, it was found that the alkaloid Phyllanthine has high binding values than Phyllantidine and the existing drug Gentamicine. The alkaloid extract of Phyllanthus emblica (amla), Phyllanthine potentially inhibits the target protein, Eae intimin (E. coli) and has the best anti bacterial activity. Hence we conclude that it shall be used as a therapeutic
agent against the uropathogenic *E. coli* but also against other enteropathogenic Gram positive bacteria which possess the virulence factor protein Eae intimin.