INTRODUCTION
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Urinary tract infections (UTIs) are of serious health concern in the Indian community and is one of the most common bacterial infectious diseases encountered in clinical practice which account for significant morbidity and mortality (Khamees, 2012). Currently, UTIs are emerging as one among the major types of body infection encountered by clinician’s in developing countries causing serious health problems to millions of people worldwide. The incidence of UTIs among general public were found to be 18/1000 person per year (Murugan et al., 2013). All age group of people including men, women and children worldwide encounter them at least once in their life time. They are the most serious bacterial infection during childhood. The episode and clinical manifestations of UTIs may greatly influenced by gender and the age group of the child. Among them, about 50% of women would have the possibilities of at least one episode during their lifetime around the world with no exception to Indian community (Koves et al., 2014). It has also been associated with high morbidity and long term complications like renal scarring, hypertension and chronic renal failure. Recent studies on pediatric UTIs in India are limited (Taneja et al., 2010).

Although several microorganisms cause UTIs, including fungi and viruses, bacteria happen to be the major causative organisms which are responsible for more than 95% of UTI cases (Jennifer et al., 2009). UTI is believed to be the most common bacterial infection with an estimated 150 million cases globally (Weichhart et al., 2008). Among the various bacterial species, *Escherichia coli* account from 80 to 85% of these infections (Vasudevan, 2014). Extra-intestinal pathogenic *E. coli* have been categorized mainly into Uropathogenic *E. coli* (UPEC), which are the leading cause of both acute and chronic urinary tract infections, often secrete a labile pore-forming toxin known as α haemolysin (HlyA) as reported by Dhakal and Mulvey (2012).

Early diagnosis and appropriate timely antimicrobial treatment are considered to be the major key factors for the elimination of uropathogens and prevention of their associated risk. Asymptomatic Bacteriuria (ASB) is the presence of significant number of bacteria (10^5 bacterial cells/ml) in a sample of mid stream urine (MSU), without the individual showing symptoms of UTI such as dysuria, frequent urination, pain during urination, cramping in the lower abdomen and burning sensation (Chukwu et al., 2010). Andrade et al. (2006) have previously reported the temporal and geographical variations in the UTI bacterial spectrum and their antimicrobial resistance. The concurrent evolution of the different classes in antimicrobial resistance among divergent bacteria complicates the therapeutic management of
UTI. Existing antimicrobial susceptibility pattern of causative uropathogens is mandatory for appropriate therapy. These escalating antimicrobial resistance cause unwanted anxiety to primary care providers who depend mostly on empirical therapy. UTI empirical antibiotic subscription should be based on the available actual antibiotic resistance data of the target bacterial population to be treated (Joshi, 2010). Since uropathogens susceptibility profile determines an efficacy of empirical therapy, surveillance studies on the current scenario of growing multiple drug resistance against the current antimicrobials under use are necessary for appropriate empirical therapy and as an alternative inhibitive agent.

Among various uropathogens, UPEC was the most frequent cause in both sexes with different age groups (Anusha et al., 2014). In a study on community acquired infections, E.coli accounted for 68% of all the positive cultures for UTIs. This was followed by Proteus mirabilis (12%), Staphylococcus aureus (10%), Enterococcus faecalis (6%) and Klebsiella aerogenes (4%). These percentages and order of uropathogens after E.coli will vary from region to region, between men and women and between children and adults (Kumar and Clark, 2005). Recent report by Murugan et al.(2012) that in gram negative organisms, E.coli (66.02%) being the predominant uropathogen followed by Klebsiella sp., (5.83%), Proteus sp., (2.26%) and in gram positive organisms, Staphylococcus sp., (12.62%), Streptococcus sp., (5.18%) and Enterococcus sp., (2.59%) in Erode district of Tamilnadu.

The problem of persistent urinary tract infection is more prominent in rural environment due to insanitary condition, lack of knowledge of personal hygiene, non availability of clinical diagnostic facilities and lack of patient’s compliance (Arjunan et al., 2010).

According to World Health Organization (WHO) 2014, the emergence of high rates of antimicrobial resistance among the causative infectious agents of common health-care associated and community-acquired infections including UTI are an escalating public health threat of considerable concern to many countries and multiple sectors. Hence, in the era of rapidly increasing antibiotic resistance, a better understanding of antimicrobial resistant causatives prevalence, its pathogenesis, inhibition underlying the virulence factors that played a critical role in clinical manifestation and their management using alternative agents is of utmost importance.

For establishment of an infection by UPEC strains, they should possess specialized several virulence factors which enables to colonize and invade the host (by their surface structures i.e., P, S, F1C and type 1 fimbriae); disrupt the host defense mechanism (by biofilm formation); injure to host tissues and or stimulate a noxious host inflammatory response (by the production of various toxins). The capacity of E.coli to produce many
virulence factors contribute to its pathogenicity (Sharma et al., 2007). Pathogenesis of UTI found to be multistep process in which it has been started with initial attachment of the UPEC cells to the host uroepithelium then it was transmitted from urethra and ascended to the bladder where it establishes type 1 fimbriae which resist normal defense mechanism of the host. The infection proceeded with the biofilm formation (intracellular bacterial communities (IBCs) by the pathogens which lead to the establishment of an infection.

Biofilm formation enhances the virulence of pathogens in various infections like dental caries, cystic fibrosis, osteo necrosis and urinary tract infection. UPEC forms intracellular bacterial communities, within the bladder epithelium. These communities may allow bacteria to subvert host defenses and form a persistent reservoir in the bladder. Biofilm formation is the core to many human infections including UTI where in soon after the surface attachment, the pathogens colonize and form a sessile biofilm community that develop on abiotic and biotic surfaces. These biofilms play an imperative role in pathogen physiology and persistence besides serving as a source of various infections. It also acts as an adhesive foundation, defense barrier which protects the embedded cells against detachment by flow shear (Jung et al., 2013). The biofilm forming ability allows these bacteria to develop additional antibiotic resistance and considerably impairs antimicrobial therapy even in infections caused by apposite antibiotics susceptible strains (Shafreen et al., 2011). Hence, once it matures this biofilm exhibit high resistance to clearance by both the host immune response and antimicrobial therapies. Also, the biofilm and their counterpart planktonic cells contrast considerably in their physiology, gene expression pattern, and even morphology. Since they are less sensitive to antimicrobial agents, controlling their growth could be a significant challenge once they are formed (Landini et al., 2010). Furthermore, this biofilm life style is associated with a high tolerance to exogenous stress, and therefore an antibiotic or other biocide treatment is more often than not ineffective to eradicate effectively (Rendueles et al., 2013). Therefore, use of antibiotics or other antimicrobial agents against a biofilm infection is not preferable. Moreover, the exposure of pathogenic bacteria to sub inhibitory concentrations of antibiotics during antimicrobial therapy normally serves as an environmental signal in itself, triggering biofilm formation. They pose a significant problem for the eradication of biofilm mediated bacterial infections in patients. Hence, detection of biocides targeting biofilm has revolutionized the research approach in medical, pharmaceutical and biosciences (Murugan et al., 2013a).

Recently it was discovered that uropathogenic *Escherichia coli* activates a complex developmental cascade upon their entry in to superficial bladder cells and divide. As the
bacteria grows, dramatic phenotypic switches result in the establishment of intracellular bacterial communities (IBCs), which progress through several stages, culminating in the formation of biofilm-like communities inside the superficial cells. Eventually, bacteria detach from the biofilm and burst out into the bladder lumen. These escaped bacteria then rebind to the epithelium and initiate another round of IBC formation. The downstream effects on this cascade might permit UPEC to evade the host immune response and persist in the urinary tract (Anderson et al., 2004). Due to the presence of biofilm communities, the cells reside very close, which leads to the transfer of resistance plasmid effectively to the neighboring cells, resulting in antimicrobial resistance.

In most of developing countries, these organisms have developed increased drug resistance over the last two decades and the management of these patients is becoming a major problem. A continuous monitoring of bacterial epidemiology and antibiotic susceptibility is crucial for clinicians in choosing appropriate empirical antibiotic for the treatment of UTI before obtaining the microbiologic results. In the current scenario, most of the strains which cause UTI were multiple drug resistant due to indiscriminate use of antibiotics. Increasing antimicrobial resistance among uropathogens poses a challenge to therapy (Zhao et al., 2009). The pattern of resistance varies geographically. In the United States, resistance increases from east to west, with the highest prevalence of multi drug resistant phenotypes on the pacific coast. The factors to be considered in the selection of appropriate antimicrobial therapy include the spectrum of activity of the agent, potential side effects, duration of the therapy, cost and pharmacokinetics. Misuse and improper prescribing policy of antibiotics causes remarkable increase in antibiotic resistant pattern among the uropathogenic E.coli isolates (Li et al., 2007). These types of resistance associated with genetic mutation and intra or inter species transfer of resistance gene through plasmids (Hughes and Datta, 1983). Frequency of UTI cases caused by multi drug resistance E.coli required strong concern of medical practitioners and health agencies. Therefore regional studies on pattern of antibiotics sensitivity are very much necessary to overcome this problem (Dash et al., 2012).

The continued increase in the multidrug-resistance (MDR) among human pathogenic microbes poses a significant challenge to pharmaceutical and biomedical sectors since they lead to a significant increase in mortality, morbidity, prolonged stay and treatment cost. Hence the present existence is the development, modification or searching a potential MDR bactericidal antimicrobial compound.
Worldwide plants are used as drugs and remedies for various diseases from time in memory. The increasing recalcitrance among pathogenic microbial infectious agents to current chemotherapeutics warrants screening of less explored medicinal plants for their control and management. The medicinal plant derivatives would serve as a prototype for the development of more effective and less toxic medicines futuristically. Moreover, the demand for plant-based therapeutics is currently on the increase globally owing to the growing recognition as natural, non-narcotic, easily biodegradable, minimum environment hazard posing products with less or no adverse side effects and easily available at affordable prices. Hence, herbal medicine still continues as the core of health care in several developing countries because of their wide biological activities, higher safety margin than the synthetic drugs and lesser costs (Shrikumar and Ravi, 2007).

Hence in the last few decades, herbal medicine is getting popularized in both developed and developing countries. India, with a rich traditional medicine system, serves as the backbone for several of these indigenous materials. Pharmacological studies have also acknowledged the value of these medicinal plants as a potential source of bioactive compounds (Prusti et al., 2008). At present, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts. Of late, improvement of human health and fitness has been centered on the use of natural products rather than commercially available antibiotics or drugs.

Terminalia chebula Retz. (Combretaceae) commonly known as black myrobalan are known for their extensive use in Ayurveda, Unani and Homoeopathic medicine and has also become a cynosure of modern medicine. The dried ripe fruits are traditionally used for the treatment of various human ailments and are known to exhibit a variety of biological activities (Tasduq et al., 2006; Chattopadhyay and Bhattacharyya, 2007; Lee et al., 2007). Numbers of reports are available on their antimicrobial activity against human pathogens. Recently Bag and Chattopadhyay (2014) reported the efflux - pump inhibition by their fruit extract compound gallotannin, 1, 2, 6 – tri ortho galloyl – beta – D – glucopyranose on multidrug resistant uropathogens.

In the present scenario, there is a growing need to develop eco friendly synthesis of nanoparticles that do not use toxic chemicals. Hence, biological methods of nanoparticles synthesis by using microorganisms (Nair et al., 2008) enzymes (Wilner et al., 2006) and plant extracts (Shankar et al., 2004) have been suggested as possible eco friendly alternatives to chemical and physical methods using plant for nanoparticles synthesis can be advantageous over other biological processes as it eliminates the elaborate process of maintaining cell
cultures. It can also be suitably scaled up for large scale synthesis of nanoparticles. It is well known that, silver is a very effective antibacterial agent and also possesses a strong activity against bacteria, fungi and viruses, although the mechanism and the manner of action are still not well known (Sharma et al., 2009). Silver in the form of various compounds have been used in Ayurveda against several bacterial infections from time immemorial (Rai et al., 2012). Nanotechnology finds its immense application in drug development and its targeted delivery (Murugan et al., 2014). Several studies reported that, the silver and silver nanoparticles may attach to the surface of the cell membrane disturbing permeability and respiration function of the cell (Kvitek et al., 2008). Many researchers reported that Ag+ ions interact with the third group in bacterial proteins, affecting the replication of DNA (Marini et al., 2007).

Today, researchers are giving much attention to nanoparticles in general and silver nanoparticles (AgNps) in particular for solving the MDR emergence problem (Gemmell et al., 2006). The synthesis of nano materials is of current interest due to their wide variety of applications in various fields including medicine (Etheridge et al., 2013). AgNps are known to inhibit the formation of biofilms (Percival et al., 2007). Shrivastava et al. (2008) proposed that these AgNps modulates cellular signaling affecting putative bacterial peptide’s phosphor tyrosine profiles which lead to bacterial growth inhibition. Green synthesized silver nanoparticles are efficient, economical and eco-friendly process which creates immense attention in the medical field for their valuable antimicrobial action (Logeswari et al., 2012).

Hence the focus of the present investigation in revolve, as the evaluation of antibacterial activity and invitro biofilm inhibitory potential of Terminalia chebula fruit extracts and their green synthesized silver nanoparticles on biofilm forming Multidrug resistant uropathogenic Escherichia coli (MDRUPEC) strains prevalent in Erode district, Tamilnadu, with an aim to develop and deliver a novel anti biofilm agent.
1.1 OBJECTIVES OF THE STUDY

- To characterize and evaluate the spectrum and antibiotic susceptibility pattern of uropathogens prevalent in Erode district, Tamilnadu.
- To ascertain the ability of biofilm formation by the multi drug resistant uropathogenic isolates.
- To screen the antibacterial activities of the selected herbal plants against uropathogens.
- To determine the inhibitory effect of *Terminalia chebula* Retz. fruit (pericarp) extract on biofilm formation.
- To determine the phytocompounds from *T.chebula* by High Performance Thin Layer Chromatography (HPTLC) method.
- To analyze the potential of the green synthesized silver nano particles of *T. chebula* and its biofilm inhibitory activity.
1.2. SCOPE AND PLAN OF STUDY

Collection of urine samples from suspected cases of patients with UTI
(From January 2009 to December 2011 including both gender from pediatric to geriatric population, at rural area, Perundurai, Erode District)

- Identification and Characterization of Uropathogens
- Antibiotic susceptibility pattern of uropathogens
- Evaluation of predominant uropathogen and its Multiple Drug Resistance (MDR) pattern

Detection of Virulence properties
1. Beta lactamase production
2. Haemolysin Production
3. Haemagglutination assay
4. Serum resistance assay
5. Osmotolerance test
6. Cell surface hydrophobicity test

Screening of selected medicinal plants
1. *Allium sativum*
2. *Coriandrum sativum*
3. *Curcuma longa*
4. *Emblica officinalis*
5. *Terminalia chebula*

Evaluation of its antibacterial activity

Molecular characterization of predominant strain
- Strain identification
- 16s RNA sequencing
- Phylogenetic Tree Construction
- NCBI Gene bank submission

Detection of various phytochemicals from efficient Plant extract
- Estimation of phytoconstituents by HPTLC
- Evaluation of biofilm inhibitory potential of identified phytocompounds by CLSM analysis

Insilico interaction of identified phytocompounds with target protein

Green synthesis of silver nanoparticles and its characterization

Detection of green synthesized silver nanoparticles biofilm inhibitory potential against identified strain