



Antibacterial activity of flower extract of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum* against Urinary tract Pathogens

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Abstract

The present study was conducted with an aim of determining antibacterial efficacy of methanol extract of flowers of *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum* belonging to the family Fabaceae. Antibacterial efficacy of flower extracts was assessed by Agar well diffusion assay against five antibiotic resistant bacteria isolated from urinary tract infection. Among flower extracts, higher inhibitory activity was shown by *C. pulcherrima* followed by *P. ferrugineum* and *D. regia*. Susceptibility to extract was recorded higher in case of Gram positive bacteria when compared to Gram negative bacteria. Among bacteria, *S. aureus* and *K. pneumoniae* were inhibited to higher and least extent respectively. The flowers of the plants selected can be the potential sources for development of therapeutically important agents active against drug resistant urinary tract pathogens. Isolation of active components from flower extracts and their inhibitory activity against urinary tract pathogens are to be carried out.

Key words: *Caesalpinia pulcherrima*, *Delonix regia*, *Peltaphorum ferrugineum*, urinary tract infection, agar well diffusion.

Introduction

The pharmacological treatment of disease began long ago with the use of plants. Plants, a key component of traditional medicine, are an important source of valuable medicines. Plants have been used to treat various types of ailments since time immemorial. Many countries of Africa, Asia and Latin America rely on traditional medicine. It is estimated that >80% of world's population relies on traditional medicine to meet primary health care needs. Throughout the world, methods of folk healing commonly utilize herbs as part of tradition. The practice of traditional medicine which is based on plants is widespread in countries such as China, India, Japan, Pakistan, Sri Lanka and Thailand. These plants are extensively used in various systems of traditional medicine such as Ayurveda, Unani, Homeopathy and Sidda. These plants also provide lead compounds for the development of pharmacologically active drugs. Many conventional drugs, for e.g., aspirin, digoxin, quinine and morphine, have been derived from plants¹⁻⁴.

Urinary Tract Infections (UTIs) are those infections caused anywhere in the urinary tract by microorganisms. These UTIs are among the most common infections in both community and hospital settings. UTIs are reported in people of all age groups in both sexes. A number of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *S. saprophyticus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Klebsiella pneumoniae* have been implicated in causing UTIs. Among these, *E. coli* is important and is isolated from >70% of cases of UTIs⁵⁻⁷. Antibiotics are commonly used to treat UTIs. However, overuse and abuse of these antibiotics often contributes to the emergence

of resistant bacterial strains. The prevalence of antibiotic resistance among these urinary tract pathogens is making treatment of UTIs more complicated⁸. Plants have been considered as a potential alternate for treatment of UTIs. Plants and plant based medicines have been used to treat urinary tract infections in various parts of the world⁹⁻¹¹. It has been shown that extracts and components of many plants possess inhibitory activity against urinary tract pathogens¹²⁻¹⁶. The present study was conducted to determine inhibitory efficacy of methanol extract of flowers of three plants viz., *Caesalpinia pulcherrima*, *Delonix regia* and *Peltaphorum ferrugineum* belonging to the family Fabaceae against antibiotic resistant urinary tract bacterial pathogens.

Material and Methods

Chemicals and media: Solvents viz., methanol and dimethyl sulfoxide (DMSO) and culture media viz., Nutrient broth and nutrient agar were obtained from HiMedia Laboratories, Mumbai.

Collection and identification of plant material: The flowers of selected plants were collected at campus of Sahyadri Science College (Autonomous) during May 2013 and identified by Mr. Gopal T.D, Assistant Professor, Department of Botany, Sahyadri Science College (Autonomous), Shivamogga. The flowers were dried under shade and powdered.

Extraction of powdered leaf and flower material: In order to extract the powdered flower materials, about 25g of dried flower materials were extracted with methanol in a Soxhlet assembly.

Later, the solvent extracts were filtered through Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and dried in desiccator⁸.

Test bacteria: The inhibitory activity of flower extracts was tested against two Gram positive bacteria viz., *Staphylococcus aureus* and *Enterococcus faecalis* and three Gram negative bacteria viz., *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* previously isolated from subjects suffering from UTIs. These bacteria were found to be multi-drug resistant (table 1).

Table-1

Urinary tract isolates and antibiotics against which the isolates are resistant

Isolate	Antibiotic
<i>E. coli</i>	Ampicillin, Norfloxacin, Amoxicillin, Cefuroxime, Cotrimazole, Cefazolin, Aztreonam, Cefpirome, Imipenem
<i>K. pneumoniae</i>	Ampicillin, Norfloxacin, Amoxicillin, Cefuroxime, Cotrimazole, Cefazolin, Aztreonam, Cefoperazone, Imipenem
<i>P. aeruginosa</i>	Gentamycin, Amikacin, Ceftazidime, Ciprofloxacin, Tobramycin
<i>S. aureus</i>	Ampicillin, Gentamycin, Norfloxacin, Penicillin
<i>E. faecalis</i>	Ampicillin, Gentamycin, Norfloxacin, Penicillin

Preparation of bacterial inocula: The test bacteria were inoculated into test tubes containing sterile Nutrient broth (HiMedia, Mumbai) and incubated at 37°C for 24 hours. The broth cultures were used for screening their susceptibility to flower extracts.

Antibacterial activity of flower extracts: Agar well diffusion method was employed to determine antibacterial activity of flower extracts against clinical isolates of UTI. The broth cultures of test bacteria were aseptically swabbed on sterile Nutrient agar (HiMedia, Mumbai) plates using sterile cotton swabs. Later, wells of 6mm diameter were punched in the swabbed plates using sterile cork borer and 100µl of flower extracts (20mg/ml of 25% dimethyl sulfoxide [DMSO; HiMedia, Mumbai]), and DMSO (25%, in sterile water) were filled in respectively labeled wells. The inoculated plates were incubated at 37°C for 24 hours in upright position and the zone of inhibition was recorded⁸.

Statistical analysis: The experiment was performed in triplicates. The results are represented as Mean ± Standard deviation (SD).

Results and Discussion

Table 2 shows the result of inhibitory effect of flower extracts. All the extracts were shown to be inhibitory to test bacteria.

Overall, Gram positive bacteria were found to be more susceptible than Gram negative bacteria. Among extracts, higher inhibition of test bacteria was observed in case of *C. pulcherrima* followed by *P. ferrugineum* and *D. regia*. *S. aureus* and *P. aeruginosa* were inhibited to higher extent among Gram positive and Gram negative bacteria respectively. *K. pneumoniae* was inhibited to least extent among test bacteria. DMSO was not found to cause inhibition of test bacteria (not shown in table).

Table-2

Inhibitory efficacy of flower extracts against urinary tract isolates

Test bacteria	Zone of inhibition in cm (Mean±SD)		
	<i>C. pulcherrima</i>	<i>P. ferrugineum</i>	<i>D. regia</i>
<i>E. coli</i>	1.5±0.1	1.4±0.1	1.3±0.0
<i>P. aeruginosa</i>	1.6±0.1	1.5±0.1	1.4±0.1
<i>K. pneumoniae</i>	1.3±0.0	1.2±0.0	1.0±0.0
<i>S. aureus</i>	1.8±0.2	1.6±0.1	1.5±0.0
<i>E. faecalis</i>	1.7±0.1	1.5±0.0	1.4±0.1

Discussion: Urinary tract infection (UTI) represents colonization of pathogens anywhere along the urinary tract. UTIs have been classified based on the site of infection (pyelonephritis [kidney], cystitis [bladder], urethra [urethritis]) and severity (complicated versus uncomplicated). UTIs are one among the most common bacterial infections affecting humans throughout their life span. These infections are the second most common infection of any organ system and the most common urological disease. UTIs are more common in females than in males^{7,17}. UTIs are acquired commonly in hospitals with an estimated prevalence of 1-10% and represent 30-40% of all nosocomial infections. The use of urinary catheter is the most important risk factor which predisposes the host to nosocomial UTIs. Instillation of catheter can lead to damage of mucosal layer, which disrupts the natural barrier and allows the colonization of bacteria. The indwelling urinary catheter is associated with the majority of these infections in particular Catheter-associated bacteriuria^{7,18,19}. The use of antibiotics is the most widely employed strategy for treatment of UTIs. But, the treatment of UTIs using antibiotics suffers from the major drawback i.e., antibiotic resistance in pathogens. Antibiotic resistance in urinary tract pathogens against commonly used antibiotics has been well reported^{20,21}.

Plants have shown to be effective against urinary tract pathogens including drug resistant strains²²⁻²⁴. Plants viz., *Barringtonia acutangula*¹³, *Cassia auriculata*²⁵, *Drynaria quercifolia*²⁶, *Terminalia chebula*²⁷, *Ballota acetabulosa*¹⁵, *Ziziphora clinopodioides*²⁸, *Avicennia marina*²⁹, *Anisomeles indica*⁸, *Teucrium polium*³⁰ have shown to exhibit inhibitory activity against urinary tract pathogens. In the present study, we have observed antibacterial effect of methanolic extract of flowers of *C. pulcherrima*, *P. ferrugineum* and *D. regia*. Extract

of *C. pulcherrima* displayed stronger inhibition of test bacteria followed by *P. ferrugineum* and *D. regia*. Similar inhibitory activity of these extracts was observed in our previous study³¹. The flower extracts were more effective against Gram positive bacteria when compared to Gram negative bacteria. In a previous study, we showed higher inhibition of Gram positive urinary tract pathogens by leaf extract of *Anisomeles indica*⁸. In another study, Pavithra *et al.*³² observed higher inhibitory activity of flower extracts of *Wendlandia thyrsoides*, *Olea dioica*, *Lagerstroemia speciosa* and *Bombax malabaricum* against Gram positive bacteria when compared with Gram negative bacteria. The lower antibacterial effect of flower extracts against the Gram negative bacteria could be attributed to the presence of an outer membrane that possess hydrophilic polysaccharides chains and forms an additional barrier³³⁻³⁴.

Conclusion

The present study showed the inhibitory potential of flower extracts of selected plants against UTI causing bacterial pathogens. The flowers of these plants can be used as potential source for the development of inhibitory agents active against antibiotic resistant bacteria. Isolation of active principles from flower extracts and determination of their antibacterial effect are to be carried out.

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