Original Research Article

**Inhibitory effect of *Anisomeles indica* Linn. against multidrug resistant urinary tract pathogens**

M.N. Vivek¹, Yashoda Kambar¹, S. Pallavi¹, T.R. Prashith Kekuda* and T.N. Ravi Kumar²

¹P.G. Department of Studies and Research in Microbiology, Sahyadri Science College campus, Kuvempu University, Shivamogga-577203, Karnataka, India
²P.G. Department of Microbiology, K.M.C, Manipal University, Manipal, Karnataka, India

*Corresponding author

**ABSTRACT**

Urinary tract infections are the infections of urinary tract and are the common infections in both community and hospital settings. The aim of the present study was to investigate inhibitory effect of methanolic extract of *Anisomeles indica* Linn. (Lamiaceae) leaf against antibiotic resistant urinary tract pathogens. Agar well diffusion assay was performed to screen the antibacterial effect of leaf extract against five isolates viz., *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Gram positive bacteria have shown higher susceptibility to leaf extract than Gram negative bacteria. *S. aureus* and *E. coli* were inhibited to high extent among Gram positive and Gram negative bacteria respectively. *K. pneumoniae* was inhibited to least extent. The plant can be a potential source for the development of agents active against urinary tract pathogens. Further studies are to be carried out to isolate active principles from the crude leaf extract and to determine their inhibitory activity against urinary tract pathogens.

**Keywords**

*Anisomeles indica*; Urinary tract infection; antibiotic resistance; Agar well diffusion.

**Introduction**

The discovery of antibiotics remains one of the significant events in the field of chemotherapy. Antibiotics have revolutionized the field of medicine and subsequent use of antibiotics saved countless individuals from infection by pathogenic microorganisms. However, microbial strains developing resistance are continuously appearing because of wide spread use which appears to be the major selective force for development of antibiotic resistance. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Enterococcus faecalis*, coliforms such as *Escherichia coli*, are among the most important antibiotic resistant microorganisms which have developed resistance against a wide range of antibiotics. These antibiotic resistant pathogens make the treatment of infections difficult. This alarming situation triggers
From ancient time, plants have been used all over the world as drugs and remedies for treatment of various kinds of ailments. The chemicals (phytochemicals) present in these plants even serve as prototype for the development of more effective and less toxic drugs (Cooke, 1976; Niemi et al., 1983; Carmeli et al., 1999; Cowan et al., 1999; Sharma et al., 2009; Demain and Sanchez, 2009; Davies and Davies, 2010; Onanuga and Awhowo, 2012; Kekuda et al., 2012; Fernandes and Dhanashree, 2013; Kekuda et al., 2013).

Anisomeles indica Linn. is an ethnomedicinally important aromatic plant and belongs to the family Lamiaceae. The plant is commonly called Catmint and is used for the treatment of various kinds of ailments in various parts of the world (Batish et al., 2007; Alagesaboopathi, 2009; Kunwar et al., 2010; Sutha et al., 2010). The crude extracts, essential oils and purified compounds from various parts of the plant (such as roots, leaves and flowers) have shown to exhibit several bioactivities such as antimicrobial (Yadava and Barsainya, 1998; Usher et al., 2010; Rao et al., 2012; Lien et al., 2013; Kundu et al., 2013), antioxidant (Huang et al., 2012; Kundu et al., 2013), attenuation of inflammation (Lien et al., 2013), analgesic (Dharmasiri et al., 2003), anti-inflammatory (Rao et al., 2009), antiviral (Alam et al., 2000), antiplatelet aggregation activity (Chen et al., 2008) and others. The root and leaf powder of A. indica when applied as mulch significantly reduced the emergence and growth of weeds of wheat crop similar to herbicide, without any negative effect on the wheat growth and yield (Batish et al., 2007). The present study was conducted with an aim to determine inhibitory activity of leaf extract of A. indica against antibiotic resistant pathogens of urinary tract infection.

Materials and Methods

Collection and extraction of plant material

The plant material was collected at college campus during September 2013. The leaves were separated, washed well in order to remove extraneous matter and dried under shade. The dried leaves were powdered mechanically and a known quantity of powdered leaf material (10g) was extracted using methanol (HiMedia, Mumbai) in Soxhlet apparatus. After extraction, the solvent extract was filtered through Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and dried in the desiccator (Kekuda et al., 2012).

Test bacteria

The antibacterial efficacy of leaf extract of A. indica was tested against two Gram positive bacteria viz., Staphylococcus aureus, Enterococcus faecalis and three Gram negative bacteria viz., Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae isolated previously from urinary tract infections. The isolates were resistant to antibiotics and Table 1 shows the name of antibiotics against which the isolates are resistant.

Antibacterial activity of extract

We employed Agar well diffusion assay to determine the potential of leaf extract to inhibit urinary tract pathogens. In brief, 24 hour old Nutrient broth (HiMedia, Mumbai) cultures of test bacteria were swabbed aseptically on sterile Nutrient
Table 1 UTI isolates and the antibiotics against which the isolates are resistant

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>Ampicillin, Norfloxacin, Amoxicillin, Cefuroxime, Cotrimazole, Cefazolin, Aztreonam, Cefpirome, Imipenem</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>Ampicillin, Norfloxacin, Amoxicillin, Cefuroxime, Cotrimazole, Cefazolin, Aztreonam, Cefoperazone, Imipenem</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>Gentamycin, Amikacin, Ceftazidime, Ciprofloxacin, Tobramycin</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>Ampicillin, Gentamycin, Norfloxacin, Penicillin</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>Ampicillin, Gentamycin, Norfloxacin, Penicillin</td>
</tr>
</tbody>
</table>

agar (HiMedia, Mumbai) using sterile cotton swabs. With the help of a sterile cork borer, wells of 6mm diameter were punched in the inoculated plates. 100μl of leaf extract (20mg/ml of dimethyl sulfoxide [DMSO]), reference antibiotic (Chloramphenicol, 1mg/ml of sterile distilled water) and DMSO (25%) were transferred into respectively labelled wells. The plates were incubated for 24 hours at 37°C. The zones of inhibition formed around the wells were measured using a ruler (Kekuda et al., 2012).

Statistical analysis

The experiment was done in triplicates. The result is taken as Mean±Standard deviation (SD).

Result and Discussion

Table 2 shows the result of antibacterial activity of leaf extract of *A. indica* against clinical isolates of UTI. A dose dependent inhibition of test bacteria was observed. Overall, the extract was found to inhibit Gram positive bacteria to high extent when compared to Gram negative bacteria. Among bacteria, highest and least susceptibility was observed in case of *S. aureus* and *K. pneumoniae* respectively. *E. coli* was inhibited to higher extent among Gram negative bacteria. DMSO was not found to inhibit any of the clinical isolates.

Urinary tract is an important system which collect, store and release urine. It include kidneys, ureters, bladder and urethra. Urinary Tract Infections (UTIs) refers infections that are caused by microorganisms anywhere in the urinary tract. These UTIs are one among the common infections in both community and hospital settings. UTIs been reported in people of all age groups in both sexes and are more common in females than in males. It can be classified as symptomatic or asymptomatic; complicated or uncomplicated. It can also be classified based on the infection site (bladder [cystitis], kidney [pyelonephritis], or urine [bacteriuria]. These infections form a serious health problem and affect millions of people globally each year. UTIs represent the leading cause of Gram-negative bacteraemia and are the most common hospital-acquired infections (Okonko et al., 2010; Beyene and Tsegaye, 2011; Humayun and Iqbal, 2012).

A number of bacteria are implicated in causing UTIs. *Escherichia coli, Klebsiella pneumoniae, Enterobacter* sp., *Pseudomonas aeruginosa, Proteus* sp., *Enterococcus faecalis, Staphylococci* and *Streptococci* common UTI causing bacterial agents. Community acquired UTIs is caused by bacteria such as *E. coli, K. pneumoniae, P. mirabilis*. 
Table 2 Inhibitory activity of extract against clinical isolates of UTI

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Zone of inhibition in cm (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extract 20mg/ml</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1.8±0.2</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>1.2±0.0</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>1.0±0.1</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>1.8±0.2</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>2.3±0.1</td>
</tr>
</tbody>
</table>

*S. saprophyticus* or *E. faecalis* whereas the hospital acquired UTIs are associated with bacteria such as *E. coli*, *P. aeruginosa*, *Proteus sp*, *Enterobacter sp.*, *Serratia sp.* or *Enterococcus sp.* Most cases of UTIs are associated with a single bacterial species, however, some may be polymicrobial in nature. The relative frequency of these urinary tract pathogens varies depending upon age, sex, catheterization, and hospitalization. Antibiotics are widely used for treating UTIs. Uncontrolled usage of these antibiotics often results in the emergence of resistant bacterial strains. The prevalence of antibiotic resistance among urinary tract pathogens is increasing worldwide and is making treatment of UTIs more complicated (Kyabaggu et al., 2007; Amin et al., 2009; Beyene and Tsegaye, 2011; Humayun and Iqbal, 2012; Shifali et al., 2012).

Studies have shown that plants and their extracts exhibit inhibitory activity against urinary tract pathogens (Peneira et al., 2004; Sharma et al., 2009; Onyancha et al., 2012; Bouabdelli et al., 2012; Kannan et al., 2012). Extract of *Barringtonia acutangula* (L.) Gaertn (Sahoo et al., 2008), *Cassia auriculata* (Thulasi and Amsaveni, 2011), *Terminalia chebula* (Bag et al., 2012), *Ballota acetabulosa* (Dulger and Dulger, 2012), *Cassia tora* (Sahu and Sinha, 2013) have been shown to possess inhibitory effect against urinary tract pathogens. In the present study, we evaluated the efficacy of leaf extract of *A. indica* to inhibit antibiotic resistant strains of urinary tract pathogens. The extract was more effective against Gram positive bacteria than Gram negative bacteria. The lower inhibitory efficacy of extract against the Gram negative bacteria could be attributed to the presence of an outer membrane that possess hydrophilic polysaccharides chains and forms an additional barrier (Lodhia et al., 2009; Nalubega et al., 2011).

In the present study, the leaf extracts of *A. indica* was shown to exhibit antibacterial activity against antibiotic resistant urinary tract pathogens. The plant can be a potential candidate for the development of bioactive agents. Further studies on isolation of active principles from the crude leaf extract and their bioactivity against UTI pathogens are to be carried out.

**Acknowledgement**

Authors are thankful to Dr. N. Mallikarjun, Associate Professor and Chairman, P.G Department of Studies and Research in Microbiology and Principal, Sahyadri Science College (Autonomous) for providing all facilities and moral support to conduct work.
References


