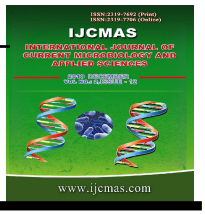
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 12 (2013) pp. 411-417**
http://www.ijcmas.com



Original Research Article

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

M.N.Vivek[1], Yashoda Kambar[1], S.Pallavi[1], T.R.Prashith Kekuda[1*] and T.N.Ravi Kumar[2]

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

A B S T R A C T

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

the search for and development of antimicrobial agents from other sources. 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 Awhowho, 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

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

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^{o}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

Isolates	Zone of inhibition in cm (Mean±SD)		
	Extract 20mg/ml	Extract 10mg/ml	DMSO
E. coli	1.8±0.2	1.5±0.2	0.0±0.0
P. aeruginosa	1.2±0.0	0.9±0.2	0.0±0.0
K. pneumoniae	1.0±0.1	0.0±0.0	0.0±0.0
E. faecalis	1.8±0.2	1.5±0.0	0.0±0.0
S. aureus	2.3±0.1	1.8±0.3	0.0±0.0

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

Alagesaboopathi, C., 2009. Ethnomedicinal plants and their utilization by villagers in Kumaragiri hills of Salem district of Tamilnadu, India. African J. Tradit. Complemen. Alterna. Med. 6(3): 222-227.

Alam, S.M., Quader, M.A., and Rashid, M.A. 2000. HIV-inhibitory diterpenoid from *Anisomelesindica*. Fitoterapia. 71(5): 574-576.

Amin, M., Mehdinejad, M., and Pourdangchi, Z. 2009. Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. Jundishapur J. Microbiol. 2(3): 118-123.

Bag, A., Bhattacharyya, S.K., Pal, N.K., Chattopadhyay, R.R. 2012. In vitro antimicrobial potential of *Terminalia chebula* fruit extracts against multidrug-resistant uropathogens. Asian Pacific. J. Trop. Biomed. 2(3): S1883-1887.

Batish, D.R., Kaur, M., Singh, H.P., and Kohli, R.K.2007. Phytotoxicity of a medicinal plant, *Anisomeles indica*, against *Phalaris minor* and its potential use as natural herbicide in wheat fields. Crop Protection. 26(7): 948-952.

Beyene, G., and Tsegaye, W. 2011. Bacterial uropathogens in urinary tract infection and antibiotic susceptibility pattern in Jimma university specialized hospital, southwest Ethiopia. Ethiopian. J. Health Sci. 21(2): 141-146.

Bouabdelli, F., Djelloul, A., Kaid-Omar, Z., Semmoud, A., and Addou, A. 2012. Antimicrobial activity of 22 plants used in Urolithiasis medicine in Western Algeria. Asian Pacific J. Trop.Disease. 2(S1): S530-S535.

Carmeli, Y., Troillet, N., Eliopoulos, G.M., and Samore, M.H. 1999. Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: Comparison of risks associated with different antipseudomonal agents. Antimicro. Agents Chemother. 43(6): 1379-1382.

Chen, Y., Lan, Y., Hsieh, P., Wu, C., Chen, S., Yen, C., Chang, F., Hung, W., and Wu, Y. 2008. Bioactive Cembrane Diterpenoids of *Anisomeles indica*. J. Natural Products. 71(7): 1207-1212.

Cooke, M.D., 1976. Antibiotic resistance among coliform and fecal coliform bacteria isolated from sewage, seawater, and marine shellfish. Antimicrobial Agents and Chemotherapy. 9(6): 879-884.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 12(4): 564-582.

Davies, J., and Davies, D. 2010. Origins and evolutions of antibiotic resistance. Microbiol. Molecular Biol. Rev. 74(3): 417-433.

Demain, A.L., and Sanchez, S. 2009. Microbial drug discovery: 80 years of progress. J. Antibiotics. 62: 5-16.

Dharmasiri, M.G., Ratnasooriya, W.D., and Thabrew, M.I. 2003. Water extract of leaves and stems of preflowering but not flowering plants of *Anisomeles indica* possesses analgesic and antihyperalgesic activities in rats. Pharma. Biol. 41(1): 37-44.

Dulger, B., and Dulger, G. 2012. Antimicrobial activity of the leaves of *Ballota acetabulosa* on microorganisms isolated from urinary tract infections. Turkish. J. Pharma. Sci. 9(3): 257-262.

Fernandes, S.C., and Dhanashree, B. 2013. Drug resistance and virulence determinants in clinical isolates of *Enterococcus* species. Indian. J. Med. Res. 137: 981-985.

Huang, H., Lien, H., Ke, H., Chang, L., Chen, C., and Chang, T. 2012. Antioxidative characteristics of *Anisomeles indica* extract and inhibitory effect of ovatodiolide on melanogenesis. International. J. Mole. Sci. 13: 6220-6235.

Humayun, T., and Iqbal, A. 2012. The culture and sensitivity pattern of urinary tract infections in females of reproductive age group. Ann. Pakistan Institute Medical Sci. 8(1): 19-22.

Kannan, R.R., Arumugam, R., and Anantharaman, P. 2012. Chemical composition and antibacterial activity of Indian seagrasses against urinary tract pathogens. Food Chem. 135(4): 2470-2473.

Kekuda, P.T.R., Manasa, M., Poornima, G., Abhipsa, V., Rekha, C., Upashe, S.P., and Raghavendra, H.L. 2013. Antibacterial, cytotoxic and antioxidant potential of *Vitexnegundo* var. *negundo* and *Vitexnegundo* var. *purpurascens*- A comparative study. Science Technol. Arts Res. J. 2(3): 59-68.

Kekuda, P.T.R., Rakesh, K.N., Dileep, N., Junaid, S., Pavithra, G.M., Gunaga, S.S., Megha, V.H., and Raghavendra, H.L. 2012. Antimicrobial and antioxidant activity of *Anaphalis lawii* (Hook.f.) Gamble. Science Technol. Arts Res. J. 1(3): 8-16.

Kundu, A., Saha, S., Walia, S., and Kour, C. 2013. Antioxidant and antifungal properties of the essential oil of *Anisomeles indica* from India. J. Med. Plants Res. 7(24): 1774-1779.

Kunwar, R.M., Shrestha, K.P., Bussmann, R.W. 2010. Traditional herbal medicine in Far-west Nepal: a pharmacological appraisal. J. Ethnobiol. Ethnomed. 6: 35.

Kyabaggu, D., Ejobi, F., and Olila, D. 2007. The sensitivities to first-line antibiotic therapy of the common urinary tract bacterial infections detected in urine samples at a hospital in metropolitan Kampala (Uganda). African Health Sci. 7(4): 214-222.

Lien, H.M., Wang, C.Y., Chang, H.Y., Huang, C.L., Peng, M.T., Sing, Y.T., Chen, C.C., and Lai, C.H. 2013. Bioevaluation of *Anisomeles indica* extracts and their inhibitory effects on *Helicobacterpylori*-mediated inflammation. J. Ethnopharmacol. 145(1): 397-401.

Lodhia, M.H., Bhatt, K.R., and Thaker, V.S. 2009. Antibacterial activity of essential oils from Palmarosa, Evening Primrose, Lavender and Tuberose. Indian. J. Pharma. Sci. 71(2): 134-136.

Nalubega, R., Kabasa, J.D., Olila, D., and Kateregga, J. 2011. Evaluation of Antibacterial Activity of Selected Ethnomedicinal Plants for Poultry in Masaka District, Uganda. Research Journal of Pharmacology. 5(2): 18-21.

Niemi, M., Sibakov, M., and Niemela, S. 1983. Antibiotic resistance among different species of fecal coliforms isolated from water samples. Appl. Environ. Microbiol. 45(1): 79-83.

Okonko, I.O., Ijandipe, L.A., Ilusanya, A.O., Donbraye-Emmanuel, O.B., Ejembi, J., Udeze, A.O., Egun, O.C., Fowotade, A., and Nkang, A.O. 2010. Detection of Urinary Tract Infection (UTI) among pregnant women in Oluyoro Catholic Hospital, Ibadan, South-Western Nigeria. Malaysian. J. Microbiol. 6(1): 16-24.

Onanuga, A., Awhowho, G.O. 2012. Antimicrobial resistance of *Staphylococcus aureus* strains from patients with urinary tract infections in Yenagoa, Nigeria. J. Pharmacy. Bio. Allied Sci. 4(3): 226-230.

Onyancha, W., Kiprono, S.J., Moindi, J., Nyakundi, E.O., and Titus, S.K. 2012. Efficacy of plant extracts against multi-drug resistant *Escherichia coli* from urinary tract infection. J. Biol. Agricult. Healthcare. 2(7): 33-38.

Peneira, R.S., Sumitha, T.C., Furlan, M.R., Jorge, A.O., and Ueno, M. 2004. Antibacterial activity of essential oils on microorganisms isolated from urinary tract infection. Revista de Saude Publica. 38(2): 326-328.

Rao, Y.K., Fang, S.H., Hsieh, S.C., Yeh, T.H., and Tzeng, Y.M. 2009. The constituents of *Anisomelesindica* and their anti-inflammatory activities. J. Ethnopharmacol. 121(2): 292-296.

Rao, Y.K., Lien, H., Lin, Y., Hsu, Y., Yeh, C., Chen, C., Lai, C., and Tzeng, Y. 2012. Antibacterial activities of *Anisomeles indica* constituents and their inhibition effect on *Helicobacter pylori*-induced inflammation in human gastric epithelial cells. Food Chem. 132: 780-787.

Sahoo, S., Panda, P.K., Mishra, S.R., Parida, R.K., Ellaiah, P., Dash, and S.K. 2008. Antibacterial activity of *Barringtonia acutangula* against selected urinary tract pathogens. Indian. J. Pharma. Sci. 70(5): 677-679.

Sahu, P.R., and Sinha, M.P. 2013. Screening of antibacterial activity of crude leaf extracts of *Cassia tora* on UTI pathogens. The Bioscan. 8(3): 735-738.

Sharma, A., Chandraker, S., Patel, V.K., and Ramteke, P. 2009. Antibacterial activity of medicinal plants against pathogens causing complicated urinary tract infections. Indian J. Pharmaceut. Sci. 71(2): 136-139.

Shifali, I., Gupta, U., Mohmood, S.E., and Ahmed, J. 2012. Antibiotic susceptibility patterns of urinary pathogens in female outpatients. North American. J. Medi. Sci. 4(4): 163-169.

Sutha, S., Mohan, V.R., Kumaresan, S., Murugan, C., and Athiperumalasami, T. 2010. Ethnomedicinal plants used by the tribals of Kalakad-Mundanthurai Tiger Reserve (KMTR), Western Ghats, Tamil Nadu for the treatment of rheumatism. Indian. J. Traditional Knowledge. 9(3): 502-509.

Thulasi, G., and Amsaveni, V. 2011. Antibacterial activity of *Cassia auriculata* against ESBL producing *E. coli* from UTI patients. Inter. J. Microbiol. Res. 2(3): 267-272.

Usher, Y.V., Tatiya, A.U., Surana, S.J., and Patil, U.K. 2010. Gas chromatography-Mass spectrometry analysis and antibacterial activity of essential oil from aerial parts and roots of *Anisomeles indica* Linn. Inter. J. Green Pharm. 4: 98-101.

Yadava, R.N., and Barsainya, D. 1998. Chemistry and antimicrobial activity of the essential oil from *Anisomeles indica* (L). Ancient Sci. Life. 18(1): 1-4.