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Antibacterial efficacy of *Pimenta dioica* (Linn.) Merill and *Anacardium occidentale* L. against drug resistant urinary tract pathogens

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ABSTRACT

The aim of the present study was to determine antibacterial effect of leaf and bark extracts of *Pimenta dioica* (Linn.) Merill (Myrtaceae) and *Anacardium occidentale* L. (Anacardiaceae) against drug resistant clinical isolates of urinary tract infection *viz*., *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis*. Agar well diffusion method was employed to assess inhibitory activity of leaf and bark extracts. Among extracts, bark extract of *P. dioica* and leaf extract of *A. occidentale* exhibited high inhibitory activity. The bark extract of *P. dioica* showed high inhibition of clinical isolates than other extracts. Among bacteria, *E. faecalis* and *K. pneumoniae* were inhibited to high and least extent respectively. The inhibitory potential of extracts could be attributed to the presence of bioactive secondary metabolites. Isolation of inhibitory principles from crude extracts and their inhibitory activity against UTI pathogens are to be carried out.

INTRODUCTION

Urinary tract represents a system that collect, store and release urine and include kidneys, ureters, bladder and urethra. Urinary Tract Infections (UTIs) are infections caused by microorganisms anywhere in the urinary tract. UTIs are one among the most common infections in both community and hospital settings and have been reported in people of all age groups in both sexes. It is more common in females than in males. It forms a serious health problem and affects millions of people worldwide each year. It remains the leading cause of Gram-negative bacteraemia. UTIs are the most common hospital-acquired infections and the common cause of bacteraemia in hospitalized patients (Okonko *et al*., 2010). UTIs are caused by a number of pathogenic microorganisms. The most common causative agents of UTIs are *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* sp., *Pseudomonas aeruginosa*, *Proteus* sp., *Enterococcus* sp., *Staphylococci* and *Streptococci*. Community acquired infection is caused by *E. coli*, *K. pneumoniae*, *P. mirabilis*, *S. saprophyticus* or *E. faecalis*, while the hospital acquired ones are *E. coli*, *P. aeruginosa*, *Proteus sp*, *Enterobacter sp*., *Serratia sp*. or *Enterococcus*. Majority of UTIs are caused by a single bacterial species. Some may be polymicrobial in nature. The relative frequency of the pathogens of UTIs differs depending upon age, sex, catheterization, and hospitalization. Antibiotics are routinely used for the treatment of UTIs.

However, uncontrolled usage of antibiotics has contributed to the emergence of resistant bacterial infections. Due to this, the prevalence of antibiotic resistance among urinary pathogens is increasing worldwide and is creating a serious problem for the treatment of UTIs (Kyabaggu *et al*., 2007; Amin *et al*., 2009; Beyene and Tsegaye, 2011; Humayun and Iqbal, 2012; Shifali *et al*., 2012). Plants have been used as medicine all over the world since long time. Plants, their extracts and the purified compounds have shown to possess a wide range of biological activities. Plants and their components have shown to possess marked antimicrobial activity (Cowan, 1999; Sharma *et al*., 2009; Vinayaka *et al*., 2009; Dulger and Dulger, 2012). Many plant species have shown to possess marked inhibitory activity against causative agents of UTIs (Peneira *et al*., 2004; Sahoo *et al*., 2008; Sharma *et al*., 2009; Vogel *et al*., 2011; Thulasi & Amsaveni, 2011; Dulger and Dulger, 2012; Kannan *et al*., 2012; Sahu and Sinha, 2013).

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In the present study, we determined antibacterial activity of leaf and bark extract of *Pimenta dioica* (Linn.) Merill (Myrtaceae) and *Anacardium occidentale* L. (Anacardiaceae) against five clinical isolates of urinary tract pathogens.

MATERIALS AND METHODS

Collection of plant materials

Leaves and barks of *P. dioica* and *A. occidentale* were collected at a place called Maragalale, Thirthahalli (Taluk), Shivamogga (District), Karnataka (State) during June 2013. The leaves and bark were washed thoroughly, dried under shade and powdered using a blender. The powdered leaf and bark materials were stored in airtight containers.

Extraction

A known quantity (10g) of powdered leaf and bark was transferred to 100ml of methanol (HiMedia, Mumbai), sonicated for 30 minutes and left at room temperature for two days. Later, the extracts were filtered through Whatman No. 1 filter paper, concentrated in vacuum under reduced pressure and dried in the desiccators (Vinayaka *et al*., 2009).

Test bacteria

In this study, two Gram positive isolates *viz*., *Staphylococcus aureus* and *Enterococcus faecalis* and three Gram negative isolates *viz*., *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* previously isolated from subjects suffering from urinary tract infections were used as test bacteria. Table 1 depicts the resistance of these isolates to various antibiotics.

Table. 1: Clinical isolates and antibiotics against which the isolates are resistant.

Isolate	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

Antibacterial activity of leaf and bark extracts

The efficacy of leaf and bark extracts to inhibit clinical isolates was tested by Agar well diffusion assay. The clinical isolates were grown in sterile Nutrient broth (HiMedia, Mumbai) for 24 hours at 37^0C. The broth cultures were aseptically swabbed on sterile Nutrient agar (HiMedia, Mumbai) plates using sterile cotton swabs. Later, wells of 6mm diameter were punched in the inoculated plates using sterile cork borer. 100µl of leaf and bark extracts (25mg/ml of 25% dimethyl sulfoxide [DMSO]) and DMSO (25%, in sterile water) were transferred into labeled wells. The plates were incubated at 37^0C for 24 hours and the zone of inhibition was recorded (Vinayaka *et al*., 2009).

Statistical analysis

The experiment was conducted in triplicates. The results are represented as Mean±Standard deviation.

RESULTS

Result of inhibitory effect of leaf and bark extracts of *A. occidentale* and *P. dioica* is shown in Table 2. Extracts were found inhibitory to all bacterial isolates but to a varied extent. Among extracts of *P. dioica*, bark extract caused higher inhibition of isolates when compared to leaf extract. In case of *A. occidentale*, high inhibitory activity was observed in case of leaf extract than bark extract. Overall, bark extract of *P. dioica* inhibited clinical isolates to high extent than other extracts. Among bacteria, *E. faecalis* and *K. pneumoniae* were inhibited to high and least extent respectively. There was no inhibition of bacteria in case of DMSO (not shown in table).

Table. 2: Inhibitory effect of leaf and bark extracts against clinical isolates.

Isolates	Zone of inhibition in cm			
	P. dioica		*A. occidentale*	
	Leaf extract	Bark extract	Leaf extract	Bark extract
E. coli	1.6±0.10	1.7±0.00	1.4±0.10	1.2±0.10
S. aureus	1.5±0.05	1.8±0.05	1.5±0.05	1.2±0.05
E. faecalis	1.8±0.10	2.0±0.10	1.6±0.00	1.2±0.05
P. aeruginosa	1.8±0.20	1.9±0.10	1.6±0.05	1.3±0.10
K. pneumoniae	1.0±0.05	1.4±0.05	1.4±0.10	1.1±0.05

DISCUSSION

The discovery of antibiotics from microorganisms is one of the most important and significant events in the field of chemotherapy. These antibiotics have revolutionized the field of medicine in many respects and their discovery and subsequent use saved countless people being infected by a number of pathogenic microorganisms. Despite the advancement in the field of chemotherapy, microbial strains resistant to one or more than antibiotics (multidrug resistant) are continuously appearing. Microorganisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis* and others are among the important antibiotic resistant microorganisms which have developed resistance against a wide range of antibiotics. This alarming situation is imposing the need for search and development of agents or drugs from natural sources. Plants have been used all over the world as drugs and remedies for various types of ailments for centuries. The phytoconstituents present in plants serve as prototype for the development of more effective and less toxic drugs (Sharma *et al*., 2009; Demain and Sanchez, 2009; Davies and Davies, 2010; Kekuda *et al*., 2013). Plants and their extracts have shown to possess inhibitory activity against urinary tract pathogens. Peneira *et al*. (2004) showed antibacterial activity of essential oils extracted from medicinal plants *Ocimum gratissimum*, *Cybopogum citratus* and *Salvia officinalis* against bacteria isolated from urine samples. Sahoo *et al*. (2008) found antibacterial activity of extracts of *Barringtonia acutangula* (L.) Gaertn against urinary tract infection causing pathogens. Sharma *et al*. (2009) showed inhibitory activity of extracts of plants such

as *Terminalia chebula*, *Ocimum sanctum*, *Cinnamomum cassia* and *Azadirachta indica* against UTI isolates. Thulasi and Amsaveni (2011) observed antibacterial activity in solvent extracts of *Cassia auriculata* against ESBL producing *E. coli* from UTI patients. Kannan *et al*. (2012) found growth inhibitory activity of Indian seagrasses against UTI causing pathogens. The extract of leaves of *Ballota acetabulosa* was found to possess inhibitory activity against bacteria and *Candida albicans* isolated from UTI (Dulger and Dulger, 2012). In another study, Sahu and Sinha (2013) showed inhibitory efficacy of crude leaf extract of *Cassia tora* against bacteria isolated from UTI patients. In the present study, we observed inhibitory potential of leaf and bark extracts of *P. dioica* and *A. occidentale* against antibiotic resistant pathogens from UTI. Among extracts of plants selected, bark extract of *P. dioica* and leaf extract of *A. occidentale* caused high inhibition of bacteria. Similar inhibitory effect *i.e*., high inhibitory activity of bark extract than leaf extract of *P. dioica* was observed against clinical isolates of *Staphylococcus aureus* and *Streptococcus mutans* in a previous study (Asha *et al*., 2013). In another study, Chaithra *et al*. (2013) found higher activity of leaf extract of *A. occidentale* against clinical isolates of *Staphylococcus aureus* and *Streptococcus mutans* than bark extract.

CONCLUSION

The leaf and bark extracts of *P. dioica* and *A. occidentale* were shown to exhibit inhibitory activity against pathogens recovered from UTI. The inhibitory activity could be ascribed to the bioactive principles present in the extracts which are to be purified from the crude extracts. The plants can be the potential candidates for the development of agents active against UTI causing pathogens.

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