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Preliminary studies on antibacterial effect of some medicinal plants against urinary tract pathogens

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Abstract

Medicinal plants have wide medicament application used to prevent and management of many ailments. These plants are used for primary health care in human communities who are deprived of modern medical care. Urinary tract infections (UTI) are most common form of bacterial infections, affecting people throughout their lifespan. Hence the present study to evaluate the antibacterial potential of folk medicine against Urinary tract infection causing pathogens. In the present study 25 urine samples were collected and from the samples totally 58 bacterial colonies were isolated, among the 58 colonies six dominant urinary tract bacterial pathogens were identified by using Biochemical and Morphological characters which were confirmed as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes*. The antibacterial activity of folk three medicinal plant extract such as *Allium sativum*, *Allium cepa* and *Zingiber officinale*, used against bacteria isolates. Compared with folk three medicinal plant extract the *Allium sativum* was effective to kill the all bacteria isolates. Hence the herbal drugs have to be subjected to extensive pharmacological, toxicological and clinical tests to confirm the prescribed status. Thus the ethnobotanical approach will be like a search for molecular diversity subjecting a wide variety of new molecules from plant sources and testing them with as many different tests as possible.

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Introduction

Urinary Tract Infection represents one of the most common diseases occurring from the neonate to the geriatric age groups encounters in medical practice today (Raju and Tiwari, 2004). It is estimated that about 35% of healthy women suffer symptoms of UTI at some stages in their life. About 5% of women each year suffer with the problem of painful urination (dysuria) and frequency (Hootan, 2003). The increasing drug resistance among these bacteria has made therapy of UTI difficult and has led to greater use of expensive broad-spectrum drugs. This resistance problem needs a re-newed effort, resulting in searching effective antibacterial agents against pathogenic microorganisms resistant to current antibiotics (Soulsby, 2005). The most common urinary tract pathogen *Escherichia coli, Pseudomonas fluorescens, Enterobacter, Enterococcus* and *Candida albicans*, are other intestinal bacteria that frequently causes UTI.

Oral antibiotics are typically used for uncomplicated infections. Intravenous antibiotics may be used for more serious infection. Among the many antibiotics commonly prescribed for UTIs are the combination to co-trimoxazole, trimethoprim, ciprofloxacin, norfloxacin, nitrofurantoin, first and second generation cephalosporins and semisynthetic penicillins with (or) without inhibitors and fosfomycin trometamol are the most commonly used antibacterial drugs in the treatment of UTI outside of the hospital. The world health organization (WHO) estimates that 4 billion people 80% of world population presently use herbal medicine for some aspect of primary health care.

Despite the existence of potent antibiotics, resistant or multiresistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs. For Centuries plants have been used throughout the world as drugs and remedies for various diseases. These drugs serve as prototype to develop more effective and less toxic medicines. Hence, an attempt has been made to evaluate antibacterial activity of folklore medicinal plants used by villages in Tamilnadu region of south India against UTI pathogens. The herbal medicine of *Allium cepa* (bulb), *Allium sativaum* (bulb and cloves) and *Zingiber officinale* (Rhizome) were used for urinary tract pathogens. *Allium cepa* is known only in cultivation, but related wild species occur in central asia. The most closely related species include *Allium vavilovii* and *Allium asarense* from Iran (Grubben and Denton, 2004). It has antibacterial activity against pathogenic organism. Garlic has been shown to have broad spectrum antimicrobial activity against many genera of bacteria, viruses, worms and fungi (Sharma, 1977). Garlic possesses diuretic, diaphoretic, and expectorant action. It is also a carminative, antispasmodic and digestant, making it useful in cases of flatulence, nausea, vomiting and indigestion (Leung and Foster, 1980). Hence the present study was conducted to evaluate the antibacterial potential of three plants extracts used in folk medicine against Urinary tract infection causing pathogens.

Materials and methods

25 urine samples were collected from Various Hospital, Thanjavur (District), India in sterile plastic universal containers and transported to laboratory in an ice cold condition by adding boric acid at a final bacteriostatic concentration of 1.8% without delay (Porter and Bordie, 1969). The isolation of Urinary tract infection causing organisms, loopful of urine sample was streaked on the nutrient agar medium and macconkey agar medium and incubated at 37°C for 24 hours (Inabo and Obanibi, 2006). After incubation the colonies were selected and characterized on the basis of morphological, cultural and biochemical characteristics (Mac Faddin, 1980) and were identified with the help of Bergey's manual of Systematic Bacteriology (Kreig and Holt, 1984).

Antibiogram of the UTI isolates was ascertained on Muller Hinton agar using disc diffusion methods (Bauer *et al.,* 1966) and CLSI standards (Clinical laboratory standards Institute 2005). 7 antibiotics most commonly used for the treatment of UTI were employed i.e. Norfloxacin 10 mcg, nalidixic acid (DNA

synthesis inhibitor) 30 mcg, Ampicillin (Cell wall synthesis inhibition) 10 mcg, Tetracycline 30 mcg chloramphenical 10 mcg, nitrofurantoin (Protein synthesis inhibitor) 300 mcg and cephotaxime 30mcg concentrations. The diameter of the zone of inhibition produced by each antibiotic disc was measured, recorded and the isolates were classified as "resistant", "intermediate" or "sensitive" based on the standard interpretation chart.

A total of three plants and their parts viz., *Zingiber officinale* (Rhizome), *Allium sativum* (Bulb and cloves), *Allium Cepa (*Bulp) were collected based on ethonomedical importance around villages area Tamilnadu, southern India. Plant materials were washed with distilled water and crushed by using pestle and morter. Then the extract was collected in a clean container (Tetyana *et al.,* 2002). Susceptibility of Urinary tract infection isolates to the extracts was determined by the disc diffusion method (Bauer *et al.,* 1966). Peteriplates containing Muller Hinton Agar medium were seeded with 24 hrs old culture of bacterial strains. The inoculums size was adjusted to achieve a final concentration of 10^8 CFU/ml by matching with 0.5 McFarland Nephlometer standards. The sterile whatman filter paper discs (5mm in diameter) impregnated with plants extract (40 mg/0.1 ml) were placed on the surface of the culture plates along with the standard antibiotic (Ofloxacin) and incubated at 37°C for 24 hrs and diameter zone of inhibition were measured in mm. The results obtained in the present investigation were subject to statistical analysis like Mean ($\bar{x}$) and Standard Deviation (SD) by Zar (1984).

Results and discussion

A total of 25 urine samples were analyzed for isolation and identification of bacterial isolates as per standard methods. Out of which 18 samples showed prominent bacterial count and remaining 7 samples showed very low bacterial count. In the present study out of 58 isolated uropathogens the most common isolates were compared with Bergey's manual of systemic Bacteriology (Table 1), Based on the results comparison bacterial colonies were confirmed us *Escherichia coli* and *Proteus vulgaris* (18%), *Klebsiella pneumoniae* (15 %) and *Staphylococcus aureus* and *Streptococcus pyogenes* (16%) and *Pseudomonas aeruginosa* (3 %) (Fig. 1).

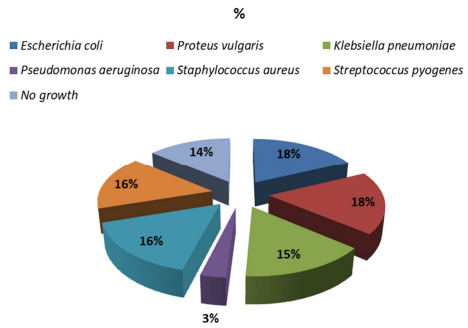

Fig. 1. Isolation of UTI pathogens from urine sample

Results of antimicrobial susceptibility test showed marked differences among urinary tract bacteria isolates in their susceptibility and resistance patterns to particular antibiotics. The bacterial isolates were generally resistant to ampicillin, cephotaxime and tetracycline. In general over all 43.3% of bacterial isolates were susceptible to norfloxacin, 66.6% to tetracycline, 50% to chloramphenicol, 83.3% cephotaxime and 66.6% showed susceptibility to nalidixic acid 83.3% (Table 2).

The antibacterial activities of different plant extract such as *Allium cepa* (Bulb) *Allium sativum* (Bulb and cloves) and *Zingiber officinale* (Rhizome) were analyzed against the isolated bacterial strains (Table 3). Maximum antibacterial activities were recorded in *Allium sativum* extract when compared than other plant extracts. Among the six bacteria strains *Allium sativum* extracts highly inhibit the growth of *Escherichia coli* (12mm). At the same time low level of growth suppression noted against *Klebsiella pneumoniae* (10mm), *Proteus vulgaris* (6mm), *Pseudomonas aeruginosa* (6 mm), *Staphylococcus aureus* (7 mm) and *Streptococcus pyogenes* (8mm).

Bacterial infection is one of the most serious global health issues in 21st century (Morris and Masterton, 2002). The emergence of bacterial resistance to antibiotics is a major health problem and therefore, it is critical to develop new antibiotics with novel

mechanism of action to overcome these problems (Wang *et al.,* 2003) plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being (Iwu *et al.,* 1999). The use of plant extracts with known antimicrobial properties can be great significance for therapeutic treatments.

Table 1. Morphological and biochemical characters of bacterial isolates

Isolated organisms	Gram staining	Motility	Shape	Indole	Methyl red	VP	Citrate	Urease	TSI	Catalase	Oxidase	Carbohydrate fermentation		
												Glucose	Sucrose	Maltose
UTB1	-	+	Rod	+	+	-	-	-	A/A	+	-	+	+	+
UTB2	-	+	Rod	-	-	+	+	-	A/A	+	+	+	+	+
UTB3	-	+	Rod	+	+	-	+/-	+	A/A	+	-	+	-	-
UTB4	-	+	Rod	-	-	-	+	+/-	K/K	-	+	+	-	+
UTB5	+	-	cocci	-	+	-	-	-	K/A	+	-	A	A	A
UTB6	+	-	cocci	-	+	-	-	-	K/K	-	-	A	A	A

+ – Positive; – – Negative; ± – Variation reaction; K/K – Alkaline slant Alkaline butt; A – Acid; K/A – Alkaline slant Acid butt

Table 2. Susceptibility of UTI isolates to various antibiotics

SL	Isolated UTI pathogens	Antibiotic resistance profile						
		Nx	T	C	A	Ce	Nf	Na
1	*Escherichia coli*	S	R	S	R	R	S	S
2	*Klebsiella pneumoniae*	R	R	S	R	R	S	R
3	*Proteus vulgaris*	R	R	R	R	S	R	S
4	*Pseudomonas aeruginosa*	S	S	S	R	S	S	S
5	*Staphylococcus aureus*	S	S	S	S	S	R	S
6	*Streptococcus pyogenes*	S	S	S	R	S	S	S

Nx- Norfloxacin , T- Tetracyclin, C- Chloramphenicol, A- Ampicillin, Ce- Cephotaxime, Nf- Nitrofurantoin , Na- Nalidixic acid, R- Resistant , S- Sensitive

Table 3. Antibacterial activities

SL	Isolated UTI Pathogens	Zone of inhibition mm in diameter			
		Standard (Ofloxacin)	*Allium sativum*	*Allium cepa*	*Zingiber officinale*
1	*Escherichia coli*	12	12	-	-
2	*Klebsiella pneumoniae*	18	10	-	-
3	*Proteus vulgaris*	11	6	-	
4	*Pseudomonas aeruginosa*	19	6	-	6
5	*Staphylococcus aureus*	24	7	-	-
6	*Streptococcus pyogenes*	20	8	-	-

The antimicrobial resistance patterns are valuables as a guide to empirical therapy and as an indicator of the dissemination of antimicrobial resistance determinants (Delappe *et al.,* 2003). The crude extract of *Allium cepa* unable to inhibit the growth of all bacterial strains. *Zingiber officinale* plant extract only inhibit the growth of *Pseudomonas aeruginosa*. Compared with three plant extract the *Allium sativum* effectively to kill the urinary tract pathogens.

Results of antibiotic susceptibility showed that nearly all the isolates were resistant against most of the antibiotics tested during the present investigation. It has been argued that there is a direct relation between the antibiotic used and the frequency and kinds of antibiotic resistant strains in human beings (Kupersztoch, 1981). The resistance to antimicrobial agents can readily be transferred among bacteria by transmissible elements-plasminds (Neu, 1994).

In this study maximum antibacterial activities were recorded in *Allium sativum* extract when compared than other plant extracts. Among the six bacteria strains *Allium sativum* extract highly inhibit the

growth of *Escherichia coli*. At the same time low level of growth suppression noted against *Klebsiella pneumonia*, *Proteus vulgaris, Staphylococcus aureus* and *Streptococcus pyogenes*. *Allium cepa* crude extract not inhibit the growth of all bacterial strains. *Zingiber officinale* plant extract only inhibit the growth of *Pseudomonas aeruginosa*. At the same time no suppression observed against other bacterial strains. Ekwenye and Elegalam, 2001 observed 9 mm zone of inhibition against *Escherichia coli* which was lesser than our studies.

Many substances may be antimicrobial, but only a few of them will be potential therapeutic agents for the simple reason that mammalian cells are more sensitive to chemical inhibition than microbial cells (Sivakumar and Alagesaboopathi, 2006). Moreover emphasized the need for toxicity testing of drugs derived from medicinal plants because the crude products obtained from such cheaper sources are often associated with a large number of compounds that have discomforting abilities (Ramdas *et al.,* 2006; Muhammad and Muhammad, 2005).

The present study has shown a spectrum of antibacterial activities, which provides a support to some traditional uses of these few medicinal plants. But the effective bimolecules which act as antibacterial have to be identified isolated and subjected to extensive scientific and pharmacological screening that can be used as sources for new drugs. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivators in the country.

Conclusion

In the present study 25 urine samples were collected from Thanjavur Various Hospitals. From the samples totally 58 bacterial colonies were isolated, among the 58 colonies six dominant urinary tract bacterial pathogens were identified by using Biochemical and Morphological characters which were named as USB1, USB2, USB3, USB4, USB5 and USB6. The isolated colonies were confirmed as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*. The antibacterial activity of folk three medicinal plant extract such as *Allium sativum, Allium* cepa and *Zingiber officinale*, used against bacteria isolates. Compared with folk three medicinal plant extract the *Allium sativum* was effective to kill the all bacteria isolates.

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