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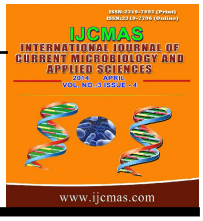

Original Research Article

Isolation, identification of microbial isolates from urinary tract infection patients and evaluation of antimicrobial activity using plant extracts

M.Priyadharsini, Sakshi Bhardwaj and E.Sheeba*

Department of Microbiology, Brindavan College,Bhoopasandra, Bangalore, Karnataka – 560094, India
**Corresponding author*

Keywords

Urinary tract infection; pathogens; antimicrobial activity; medicinal plants; MIC

A B S T R A C T

Urinary tract infection (UTI) is one of the most common microbial infections affecting all age groups across the life span. The present study was aimed to gain knowledge about the type of pathogens responsible for urinary tract infections and evaluation of antimicrobial activity of various plant extracts against the pathogens. 60 samples were collected and processed. In that 48 samples were confirmed as urinary tract infection. Bacterial species isolated from urine samples were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus sp.*, From the UTI sample *Candida albicans* also isolated. Present study showed that *E.coli* and *Staphylococcus aureus* isolates were the predominant pathogens. Four medicinal plants were selected to evaluate antimicrobial activity of methanol, ethanol and chloroform extracts against 7 isolates of major urinary tract pathogens by well diffusion method and MIC. The methanol and ethanol extract of *Carica papaya* seeds, *Ocimum santum* leaves, ethanol extract of *Azadirachta indica* leaves, and methanol and chloroform extract of *Hemidesmus indicus* root showed maximum activity against isolated pathogens. The main purpose of the study was to identify the pathogens from urinary tract infection patients and detect the effectiveness of selected species of plant extracts against these pathogens.

Introduction

Urinary tract infections (UTI) are serious health problems affecting millions of people each year. They are the second most common type of infections in the body (Stamm *et al.*, 2001). This problem occurs more often in women than men because a woman's urethra is shorter. The short urethra makes it easier for bacteria from the anus or genital area to reach the bladder. Patient with catheter or patients suffering from complaints of prostatitis are also prone to UTI. Transmission occurs in four ways; namely through sexual intercourse, from mother to the foetus via placenta, through poor personal hygiene, and via communal sponge and towel usage

(Onifade *et al*., 2011). The most common symptoms are burning with urination and having to urinate frequently (or an urge to urinate) in the absence of vaginal discharge and significant pain. For patients who have frequent UTIs, their bacteria may become resistant to antibiotics over time, making careful selection of antibiotic and the full course of treatment essential. In the last three decades, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing urinary tract infections (Tenever and McGowan, 1996; Hryniewicz *et al*., 2001; Kurutepe *et al*., 2005).

Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry and it is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. Different extract of Sarsaparilla (*Hemidesmus indicus)*, papaya seeds (*Carica papaya*), neem (*Azadirachta indica*) & tulsi (*Ocimum sanctum*) showed antibacterial activity against some microbes.

Hemidesmus indicus is a species of plant slender, laticiferous, twining, sometimes prostrate or semi-erect shrub. Roots are woody and aromatic. It is occurs over the greater part of India, from the upper Gangetic plain eastwards to Assam and in some places in central, western and South India. Roots of *H. indicus* are reported to contain chemical constituents like - an essential oil containing 80% of 2-hydroxy 4-methoxy benzaldehyde, a ketone, fatty acids, saponin, tannins, resinal fractions, resin acids, sterols, sitosterol, stigmasterol and sarsapic acid. Hemidesmin 1, hemidesmin 2, alpha-amyrin, beta-amyrin, lupeol and 2-hydroxy-4-methoxy benzoic acid have been isolated and identified from roots of *H. indicus*.

Papaya juice has an *in vitro* antiproliferative effect on liver cancer cells, possibly due to lycopene or immune system stimulation. Papaya seeds might contain antibacterial properties.

Neem (*Azadirachta indica*) is a tree in the family Meliaceae. Products made from neem have been used in India for over two millennia for their medicinal properties: they are said to be antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative.

Tulsi (Holy Basil) is a traditional plant considered sacred by the Hindus. Oil extracted from leaves of this plant possesses significant insecticidal properties .*Ocimum sanctum* has been extensively studied for therapeutic potentials in various areas like immuno-stimulation, anticancer antioxidant, as adjuvant to radiotherapy, antiulcer, analgesic and antidiabetic.

Materials and Methods

Study population

The study population was drawn from patients attending Kempegowda Institute of Medical Sciences (KIMS), Bangalore. Patients on antibiotic therapy were excluded from the study.

Isolation and identification of UTI isolates

60 urine samples from UTI patients were collected from pathological laboratory. For the isolation of UTI causing strains, loop

full of urine sample was streaked on to Nutrient agar and Mac Conkey agar plate and incubated at 37˚C for 24hrs. Next day individual colonies were selected and identified on the basis of morphological, cultural and biochemical characteristics.

Identification of organism

To check morphological characteristics, Gram-staining, capsule staining and motility test were performed. To check the growth pattern, different media including Nutrient agar, MacConkey agar, Eosine Methylene Blue agar, Mannitol Salt agar, Citrimide agar, Bi.G.G.Y agar (Bismuth Glycine Glucose Yeast agar) and Blood agar base supplemented with 5% sheep blood were used. For biochemical characteristics, sugar fermentation (lactose, glucose, mannitol, maltose, sucrose and xylose), TSI, IMViC (indole, MR, VP, citrate) oxidase, catalase and nitrate tests were performed.

Maintenance of clinical isolates

Stock cultures were maintained in vials on nutrient agar and stored at 4˚C for further study.

Plant materials

The leaves of *Azadirachta indica* and *Ocimum sanctum*, seeds of *Carica papaya* and roots of *Hemidesmus indicus* were used for antimicrobial study.

Preparation of extract

For this purpose, shade dried powdered of plant materials were used for extraction with different solvents (ethanol and methanol were used as a solvent to extract the bioactive compounds of *Carica papaya* seeds and *Ocimum sanctum* leaves, chloroform and methanol were used as a solvent to extract the bioactive compounds of *Hemidesmus indicus* and ethanol were used as a solvent to extract the bioactive compounds of *Azadirachta indica*) by using Soxhlet apparatus.

Antimicrobial Activity Test

Microorganisms used for antimicrobial activity

Staphylococcus aureus, Escherichia coli, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus sp.*, *Staphylococcus epidermidis* and *Candida albicans*.

Determination of the antimicrobial activity of medicinal plants

Assay of antimicrobial activity of medicinal plant extract was done by agar well diffusion method and MIC.

Well Diffusion Method

The Mueller Hinton agar plates were prepared and test microbial strains were swabbed on the MHA plates using sterile cotton swabs. Five wells were made with cork borer. Different concentrations (1000µg/ml - 5000µg/ml) of leaf extract, root extract and seed extract were poured in the wells. Then the plates were incubated at 37˚C for 24 – 48 hours. After incubation period, zone of inhibition were measured and recorded. Control plates were prepared without plant extract using only different solvents. The tests were performed in duplicates for each microorganism evaluated and the final results were presented as the arithmetic average. The inhibition zones were measured in millimetres. The results obtained from leaf extract, root extract and seed extracts were compared to know the effectiveness of leaves, roots or seeds against pathogens.

Minimal inhibitory concentration (MIC)

The MIC estimated and serial dilutions of broth and various concentrations of herbal extracts were made to 3.0 ml in test tube. Then cultures were added. The test tubes were incubated at 37˚C for each type of microbial culture. The lowest concentration of the crude drug that inhibited the growth of microorganisms completely was considered as MIC.

Results and Discussion

In this study, 48 patients out of 60 were showed to be urine culture positive. There were 33(69%) females and 15(31%) males in patients with urine positive culture. On the Nutrient agar plate and Mc conkey agar plate the colonies were isolated and identified. Age group of 21 – 30 were showed maximum infection. Female patients were more when compared with male patients (Table – I). Percentage of *E.coli* was present in maximum percentage in the urine samples and minimum percentage was *Candida albicans* (Figure - 1). Microorganisms isolated from urine samples were *E.coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus* sp. Candida albicans identified with the help of staining and germ tube test. The pathogens identified subcultured on nutrient agar slants for further use.

Antimicrobial activity of different plant extracts against pathogens

Carica papaya, Hemidesmus indicus, Azadirachta indica , Ocimum sanctum showed significant antimicrobial activity against the 7 different pathogens of UTI patients. All the extracts were assessed for their antimicrobial properties by agar well diffusion method and MIC also performed to see the minimum concentration of plant extract which inhibit the growth of pathogens completely.

In our study, we have achieved isolation, identification of pathogens from urine samples with help of normal media, differential media, and selective media and biochemical tests. Evaluation of antimicrobial activity also performed with different plant extracts such as methanol and ethanol extract of *Carica papaya* seeds, *Ocimim santum* leaves, ethanol extract of *Azadirachta indica* leaves, methanol and chloroform extract of *Hemidesmus indicus* root.

The study implicated 7 microorganisms as possible aetiological agents of UTI cases observed. These organisms were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus sp., Candida albicans*. They are common causative agents of urinary tract infection mentioned by other researchers also.(Meers *et al* 2002, Kolawole *et al*, 2009, Mansour *et al*, 2009). The uropathogens identified in our study are similar to those of many other studies conducted in different countries either in the region or internationally. (Astal *et al*, 2002). However different results have been reported. The similarities and differences in the type and distribution of uropathogens may results from different environmental conditions and host factors, and practices such as healthcare and education programmes, socioeconomic standards and hygiene practices in each country.

In the present study, most common

causative organisms of UTI was *Escherichia coli* (31.25%) followed by *Staphylococcus aureus* (16.66%), *Klebsiella pneumoniae* (14.50%) *Pseudomonas aeruginosa* (12.50%), *Streptococcus sp.* (10.41%) and least common pathogens in *Staphylococcus epidermidis* (8.33%) and fungi *Candida albicans* (6.25%). This higher prevalence of *E.coli* may be due to the faecal contamination, the prediction of the organisms from the toilets and the shortness of the female urethra. Other research works also supporting the same result (Foxman B, 2003). The prevalence of Gram-positive cocci was not high in some studies (Mansour *et al*, 2009), this is dissimilar to our studies in different countries. *Enterobacteriaceae*, are the commonest organisms isolated from UTI with uncomplicated cases.*E.coli* is the most frequent etiological agent causing community and hospital acquired UTIs. Infection of the urinary tract due to *Candida albicans* is an uncommon but well-described complication of modern therapeutics. From the urine *Candida albicans* was isolated.

In the study, the frequency of UTI was greater in women as compared to men. (68.75%) of the patients were females and (31.25%) were males principally owing to anatomic and physical factors. This is in agreement with other reports which stress that UTI is prevalent in females than in male during youth and adulthood. That conclusion supported by other research works done in international level. (Abu, 2000). In the present research work, the cases of UTI chances examined based on age and gender. It was observed that majority of the positive cases fall between ages 21to 30 years. In the recent years development of multi drug resistance in the pathogenic microorganisms is high.

During this study 4 plants were selected which were used for the treatment of UTI. Undoubtedly the plant kingdom still holds many species of the plant containing substances of medicinal values that are yet to be discovered, though large numbers of plants are constantly being screened for this antimicrobial properties but more pharmacological investigation is necessary.

We found that out of 4 plants extract the maximum zone of inhibition was observed in the chloroform extract of *Hemidesmus indicus* against *Candida albicans* (28 mm), *Staphylococcus aureus* (16 mm), *Escherichia coli* (25 mm) respectively.

Azardichata indica ethanol extract was also effective against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *Carica papaya* ethanol and methanol extracts were active against all bacteria except *Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans*. Methanol extract of *Ocimum santum* and *Hemidesmus indicus* was also comparatively effective. *Ocimum santum* ethanol extract showed effective zone of inhibition against *Staphylococcus aureus, Klebsiella pneumoniae and Candida albicans.*

A urinary tract infection represents one of the most common diseases occurring today. UTI is most serious global health issues in 21st century. Recurrent urinary tract infections (UTIs) present a significant

Table.1 The distribution of UTI patients in relation to their age group

Sl.No	Age group(years)	No. of Male	No. of Female
1	1-10	1	0
2	11-20	0	4
3	21-30	4	12
4	31-40	3	9
5	41-50	2	4
6	51-60	3	3
7	61-70	2	1

Table.2 Percentage of Gram positive and Gram negative Bacteria isolated from UTI Patients

Bacterial isolates	Colony Morphology	Total organisms	Total % of UTI isolates	Individual % of G(-) &G(+) UTI isolates
Gram(-)ve		**28**		
E.coli	Small, circular, slightly raised, smooth	15	31.25%	53.5%
K.pneumoniae	Circular, mucoid convex, small colonies, capsulated	7	14.5%	25%
P.aeruginosa	Small, rough colony, flat edges	6	12.5%	21.42%
Gram(+)ve		**17**		
S.aureus	Circular, pinheaded colonies, convex with entire margins	8	16.66%	47%
S.epidermidis	Whitish, circular, pinheaded colonies, convex with entire margins	4	8.33%	23.5%
Streptococcus sp.	Circular with entire margin, often raised with depressed centres	5	10.41%	29.41%

Table.3 Biochemical tests for the identification of microbial isolates

Bio chemical test	*E.coli*	*S.aureus*	*S.epidermidis*	*Streptococcus sp.*	*K.pneumoniae*	*P.aeruginosa*
Indole	+	-	-	+	-	-
MR	+	+	-	-	-	-
VP	-	+	+	-	+	-
Citrate	-	-	-	-	+	+
Nitrate	+	+	+	-	+	+
Urease	-	-	+	+	+	-
TSI	A(slant, butt) H_2S^-	A(slant, butt) H_2S^-	A butt, alkaline slant H_2S^-	A slant H_2S^-	A(slant, butt) H_2S^-	Alkaline slant and butt
Catalase	+	+	+	-	+	+
Oxidase	-	-	-	-	-	+
Glucose	AG	A	A	A	AG	A
Lactose	AG	A	A	A	AG	-
Xylose	A	-	-	-	-	-
Mannitol	AG	A	-	-	AG	-
Maltose	-	A	A	-	-	-
Sucrose	-	A	A	A	AG	-
Motility	+	-	-	-	-	+

(AG – Acid, Gas produced)

problem for women and a challenge for the doctors who care for them. Correct identification of pathogens from the clinical samples also important.

Antimicrobial susceptibility patterns varied in isolates from different categories. Bacterial pathogens have evolved numerous defence mechanisms against antimicrobial agents; hence resistance to old and newly produced drugs is on the rise. The phenomenon of antibiotic resistance exhibited by the pathogenic microorganisms has led to the need for screening of several medicinal plants for their potential antimicrobial activity. Thus the present study was undertaken to investigate the antimicrobial activity of 4 medicinal plants against UTI causing isolates.

It is concluded that the *Carica papaya*, *Ocimum santum*, *Hemidesmus indicus* and *Azadirachta indica* showed maximum activity against uropathogens, and it can be used as a therapeutic agent. This significant study will aid the clinician to prescribe adequate treatment for urinary tract infection in traditional way. Further purification and analysis are necessary to determine the specific activity of these plant extracts.

Most of the antibiotics usually used for the treatment of UTI are resistant. It is now very necessary to develop new antimicrobials and therapeutic agents having high effectiveness with no side effects, easy availability and less expensive. The present study helps to

understand the presence of bioactive compounds in the plants and application of these plants in the pharmaceutical industry in future.

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