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ANTIBACTERIAL ACTIVITY OF SELECTED MEDICINAL PLANTS AGAINST URINARY TRACT INFECTION CAUSING CLINICAL ISOLATES

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Abstract

Eighteen urine samples were collected from Government hospital, Kurinjipadi, Cuddalore district, Tamil Nadu. Twelve isolates were screened from urinary tract infection and were identified as *E coli, Pseudomonas aeruginosa, Proteus sp and Staphylococcus aureus*. The isolated pathogens were tested for their antimicrobial susceptibility using 10 antibiotics. *Pseudomonas aeruginosa* exhibited maximum susceptibility to Chloramphenicol (33mm) and resistant to Ampicillin, Penicillin and Erythromycin. *E. coli* exhibited maximum susceptibility to Chloramphenicol (36mm) and resistant to Penicillin, Ampicillin and Erythromycin. *Staphylococcus aureus* exhibited maximum susceptibility to Gentamycin (35mm). *Proteus sp* showed maximum sensitivity to Ciprofloxacin (25mm) and resistant to Penicillin. Four different medicinal plants such as *Abutilon indicum* (leaf), *Cyanodon dactylon* (leaf), *Ocimum sanctum* (leaf) and *Ricinus communis* (seed) were used. The plant materials were processed and made into a fine powder and the extract was prepared using different solvents such as ethanol, methanol, acetone and water. *Abutilon indicum* leaf, methanol extract showed maximum inhibition against *E coli* (24mm) and *Pseudomonas aeruginosa* (24mm). *Cyanodon dactylon* leaf, ethanol extract showed maximum inhibition against *Pseudomonas aeruginosa* (21mm). *Ocimum sanctum* leaf, acetone extract showed maximum inhibition against *P aeruginosa* (30mm). *Ricinus communis* seed, ethanol extract showed maximum inhibition against E coli (30mm). *Ocimum sanctum* and *Ricinus communis* extracts showed good inhibition against UTI causing bacterial isolates.

Keywords: Urinary tract infection, solvents, *Abutilon indicum, Cyanodon dactylon, Ocimum sanctum* and *Ricinus communis*.

INTRODUCTION

Urinary tract infection is one of the most prevalent extra intestinal bacterial infection (6). The urinary tract consist of kidneys, ureters, bladder and urethra. More than 95% of UTI are caused by single bacterial species. *E coli* which is the most frequently infecting organism. Worldwide about 150 million people are diagnosed with UTI each year. Majority of urinary tract infections are not life threatening and do not cause any irreversible damage (6). UTI can be defined by the presence of significant quantity of bacteria in the urine.

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UTI that affect any part of urinary tract.When bacteria get enter into the bladder or kidney, it multiplies in the urine and cause UTI (4). The incidence of acute uncomplicated urinary tract infection is estimated to exceed 0.5 % episodes per annum among women between 18-30 years (9).It is reported that about 35% of healthy women suffer symptoms of urinary tract infection and about 5%of women each year suffer with the problem of painful urination (dysuria) and frequency .The incidence of UTI is greater in women when compared to men (5). Urethritis, Cystitis and Pyelonephritis are the types of UTI infection.

Symptoms of UTI infection are painful urination, frequent urination or urge to urinate while those of pyelonephritis include fever and flank pain. UTI occurs when a significant number of microorganism (10^5 cells/ml) present in urine from catheter specimen. Uropathogenic *E coli* (UPEC) are responsible for approximately 90% of UTI. Most of the cases of UTI occurs as community acquired infection (1). Herbal plants and their preparation have been documented in ancient Indian literature and found to be effective in treatment of disease.

The research work is focused to find out the antibacterial activity of medicinal plants against UTI causing bacterial isolates. The medicinal plants used for the present study were *Abutilon indicum* (Thuthi), *Cyanodon dactylon* (Arugampul), *Ocimum sanctum* (Thulasi) and *Ricinus communis* (Castor oil plant).

Materials and Methods

Sample collection from UTI patients: Eighteen urine samples were collected from Government hospital, Kurinjipadi, Cuddalore district,Tamil Nadu. The samples were collected from patients clinically suspected to have UTI infection. The midstream urine was collected in a sterile container and was aseptically transferred to the laboratory. The urine samples were collected from patients with symptoms such as frequency of urination, pregnant women, patients with kidney stones, in catheterized patients and with bladder infection.

Processing of urine sample: A loopfull of urine sample was inoculated into the media such as Eosin Methylene Blue agar (EMB), Cetrimide agar, Mannitol Salt Agar (MSA) and MacConkey agar plates. The plates were then incubated at 37°C for 24 hours. After incubation the isolates were purified and identified.

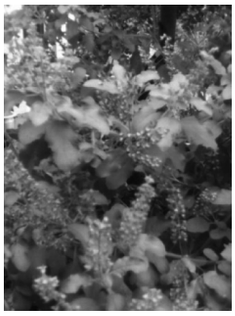
Identification of bacteria from urine sample: The isolates were characterized based on morphological and biochemical characteristics. Morphological characteristics including Gram staining, and motility and biochemical reactions such as Indole, Methyl Red, Voges – Proskauer, Citrate utilization, Triple Sugar Iron agar, Urease, Catalase and Oxidase test.

Antimicrobial susceptibility test: Antimicrobial susceptibility test was performed by the Kirby bauer method (2) against 10 antibiotics such as Ampicillin (10mcg), Amikacin (30mcg), Chloramphenicol (30mcg), Ciprofloxacin (10mcg), Erythromycin (15mcg), Gentamycin (10mcg), Kanamycin (30 mcg) , Penicillin (10 units) Streptomycin (10 mcg) and Tetracycline(30 mcg).

Collection of herbal plants: Four different medicinal plants such as *Abutilon indicum* (leaf), *Cyanodon dactylon* (leaf), *Ocimum sanctum* (leaf) and *Ricinus communis* (seed) were collected from Kurinjipadi, Cuddalore district, Tamilnadu.

Medicinal Plants

Abutilon indicum



Ocimum sanctum

Ricinus communis

Cyanodon dactylon

Processing of Plant Samples

The plant materials were collected and cleaned with water to remove the soil and dust particles on the surface of the samples. The leaves and seed samples were shade dried for 10-15 days and made into powder and stored in clean airtight plastic containers for further use (8).

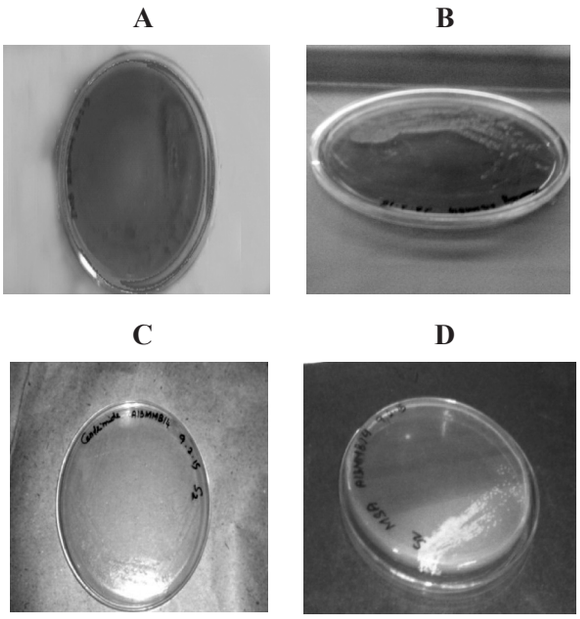
Preparation of herbal extracts: The powdered plant samples were mixed with solvents like ethanol, methanol, acetone and water individually. Then they were kept at room temperature for 72 hours. Each mixture was stirred every 24 hours using sterile glass rod. The extract was filtered using Whattman no.1 filter paper. Extraction procedure was done further twice for complete extraction of the bioactive compounds. The filterate was collected in a separate beaker and concentrated by evaporating the solvents(7).

Antibacterial activity of medicinal plant extracts: The solvent extracts (Ethanol, methanol, Acetone and water) of Leaf samples of *Abutilon indicum, Cynodon dactylon, Ocimum sanctum* and seed sample of *Ricinus communis* were prepared and tested against isolated and identified UTI pathogens. Muller Hinton agar was poured into the sterile Petri plates and allowed to solidify. The test organisms were swabbed on Muller Hinton agar plates. The sterile disc loaded with 10 µl of plant extracts are placed on the swabbed plates at equal interval. Then the plates were incubated at 37° C for 24 hours. The diameter of inhibition zone was measured in mm after 24 h.

Results and Discussion

Identification of bacterial pathogens: Bacterial isolates screened from 18 mid stream urine samples were identified as *E coli, Pseudomonas aeruginosa, Proteus sp* and *Staphylococcus aureus.* Jagadeesan *et al* (4) collected 40 urine samples for isolation of predominant bacterial pathogens. Most of the isolates were identified as *E coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus viridans*.

UTI Pathogens in Specific Media



A-*E coli* on Eosin Methylene Blue agar

B-*E coli* on MacConkey agar

C-*Pseudomonas aeruginosa* on Cetrimide agar

D-*Staphylococcus aureus* on Mannitol salt agar

Morphological and Biochemical charactarisation of UTI causing Bacterial Isolates

S. No	Test	*E coli*	*Pseudomonas aeruginosa*	*Proteus sp.*	*Staphylococcus aureus*
1.	**Morphological characteristics**				
	Gram staining	Gram negative rod	Gram negative rod	Gram negative rod	Gram positive cocci
2.	Motility test	Motile	Motile	Motile	Non–motile
1.	**Biochemical characteristics**				
	Indole test	Positive	Negative	Positive	Negative
2.	Methyl red test	Positive	Negative	Positive	Positive
3.	Voges –Proskauer test	Negative	Negative	Negative	Positive
4.	Citrate test	Negative	Positive	Negative	Negative
5.	Triple sugar iron agar test	Acid butt, Acid slant	Alkaline butt, Alkaline slant	Acid butt, Acid slant	Acid butt, Acid slant
6.	Urease test	Negative	Negative	Positive	Positive
7.	Catalase test	Positive	Positive	Positive	Positive
8.	Oxidase test	Negative	Positive	Negative	Negative

Antimicrobial Susceptibility Pattern of Urinary Tract Infection Causing Bacterial Pathogens

E coli showed maximum sensitivity to Chloramphenicol (36mm) and resistant to Penicillin, Ampicillin and Erythromycin. *Pseudomonas aeruginosa* showed maximum sensitivity to Chloramphenicol (33mm) and resistant to Ampicillin, Penicillin and Erythromycin. *Staphylococcus aureus* showed maximum sensitivity to Gentamycin (35mm) and resistant to Ampicillin and Penicillin. *Proteus sp* showed maximum sensitivity to Ciprofloxacin (25mm) and resistant to Penicillin. Chakraborty *et al* (3) investigated that antibiotic resistance is a major clinical problem in treating infectious disease caused by *E coli*. The resistance to the antimicrobials has increased over the years and normal intestinal microbial flora became a reservoir for resistance genes.

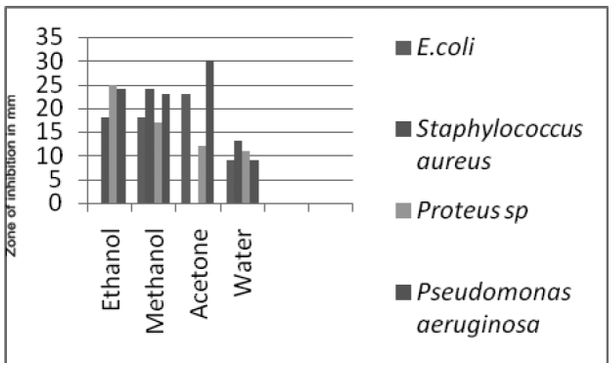
Antibacterial Activity of *Abutilon Indicum* Leaf Extract Against UTI Causing Bacterial Isolates

The ethanol extract of *Abutilon indicum* showed maximum inhibitory activity against *Staphylococcus aureus* (22mm) and moderate activity against *Proteus sp* (17mm), *Pseudomonas aeruginosa* (15mm) and *E coli* (12mm).The methanol extract of *A indicum* showed maximum activity against *E coli* (24mm) and *Pseudomonas aeruginosa* (24mm), least activity against *Proteus sp* (9mm) and no activity against *S aureus*. The acetone extract of *Abutilon indicum* showed maximum activity against *S aureus* (20mm) and no activity against *E coli*, *Proteus sp* and *P aeruginosa*.The water extract of *Abutilon indicum* showed moderate activity against *E coli* (16mm) and *Proteus sp* (12mm).Least activity against *Pseudomonas aeruginosa* (10mm) and no activity against *S aureus*. Among the 4 solvent extracts of *Abutilon indicum* leaf, methanol extract showed maximum inhibition against *E coli* (24mm) and *Pseudomonas aeruginosa* (24mm).

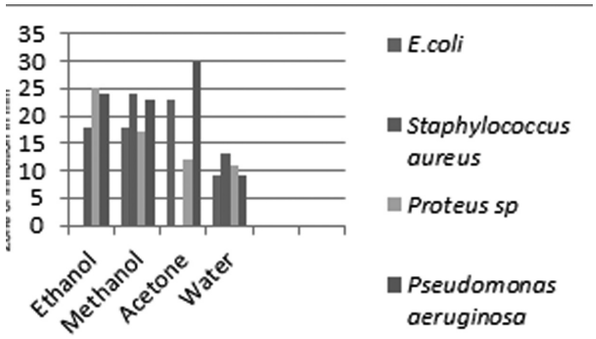
Antibacterial Activity of *Cyanodon Dactylon* Leaf Extract Against UTI Causing Bacterial Isolates

The ethanol extract of *Cyanodon dactylon* showed maximum inhibitory activity against *P aeruginosa* (21mm) and moderate activity against *Proteus sp* (18mm). No activity was noted against *E coli* and *S aureus*. Methanol extract of *Cyanodon dactylon* showed maximum activity against *P aeruginosa* (20mm) and moderate activity against *S aureus* (19mm). No activity was noted against *Proteus sp* and *E coli*. Acetone extracts of *Cyanodon dactylon* showed moderate activity against *P aeruginosa* (18mm) and no activity against *E coli*, *Proteus* sp and *S aureus*. Water extracts of *Cyanodon dactylon* showed moderate activity against *Proteus sp* (11mm), *S aureus* (11mm) and *P aeruginosa* (10mm). No activity against *E coli* .Among the 4 solvent extracts of *Cyanodon dactylon* leaf, ethanol extract showed maximum inhibition against *Pseudomonas aeruginosa* (21mm).

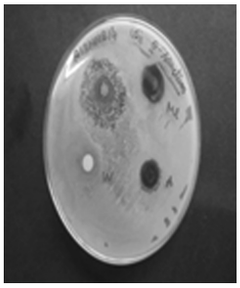
Antibacterial Activity of *Ocimum Sanctum* Antibacterial Activity of *Ricinus Communis* Seed Extract Against UTI Isolates Leaf Extract Against UTI Isolates



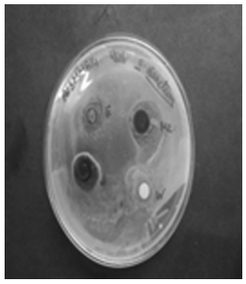
Antibacterial Activity of Ricinus Communis Seed Extract Against UTI Isolates



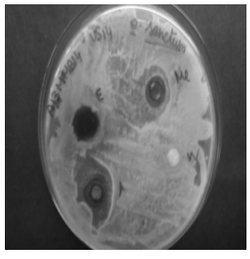
Activity of Ocimum sanctum leaf extract against UTI causing bacterial pathogens



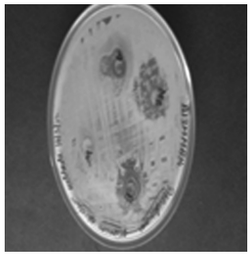
Proteus sp



Staphylococcus aureus



E coli



Pseudomonas aeruginosa

E-Ethanol A-Acetone M-Methanol W-Water

Sharma *et al* (10) investigated the antibacterial activity of 15 medicinal plants used by tribals against UTI. The antibacterial activity of aqueous, ethanol and acetone extracts of medicinal plants were tested against UTI isolates i.e. *Proteus mirabilis , Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Enterobacter cloacae and Providencia pseudomallei*. The crude extracts of the selected plants especially the acetone and ethanol extracts exhibited significant activity against UTI pathogens.

CONCLUSION

The research findings established the potential of *Abutilon indicum* (leaf), *Cyanodon dactylon* (leaf), *Ocimum sanctum* (leaf) and *Ricinus communis* (seed) as an effective antimicrobial agent for UTI.

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