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Isolation and antibacterial susceptibility testing of multi drug resistant *Pseudomonas aeruginosa* causing urinary tract infections

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ABSTRACT

The present study was carried out to evaluate the antibacterial activities ofethanolicand aqueous extracts of Kigelia africana (Bignoniaceae) and Carica papaya (Caricaceae) against multi drug resistant Pseudomonas aeruginosa UTI causing pathogen. The bacterial sp was isolated from urine sample of UTI infected patient and identified with the help of colony characterization, gram staining and biochemical testing. In our study both extracts shows antibacterial activity against Pseudomonas aeruginosa.

Keywords: Antibacterial activity, UTI, *Kigelia africana* (Bignoniaceae), *Carica papaya* (Caricaceae), *pseudomonas aeruginosa.*

INTRODUCTION

Urinary tract infection (UTI) is the second most common infectious presentation in community medical practice. Worldwide, about 150 million people are diagnosed with UTI each year, and UTI are classified as uncomplicated or complicated [1].Uncomplicated UTIs occur in sexually active healthy female patients with structurally and functionally normal urinary tracts. Complicated UTIs are those that are associated with co morbid conditions that prolong the need for treatment or increase the chances for therapeutic failure. These conditions include abnormalities of the urinary tract that impede urine flow, the existence of a foreign body (e.g., indwelling catheter, stone), or infection with multidrug resistant pathogens. UTIs in male patients are considered complicated. Despite involvement of the upper urinary tract, pyelonephritis can be considered uncomplicated when it occurs in a healthy patient [2].Urinary tract infection may involve only the lower urinary tract or both the upper and the lower tracts. The term cystitis has been used to describe the syndrome involving dysuria, frequency, and occasionally suprapubic tenderness. Acute pyelonephritis describes the clinical syndrome characterized by flank pain or tenderness, or both, and fever, often associated with dysuria,

urgency, and frequency [3]. *Klebsiella, Staphylococci,* Enterobacter, *Proteus, Pseudomonas*, and Enterococci species are more often isolated from patients. Urinary Tract Infections (UTIs) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is perhaps the single most common bacterial infection of mankind [4]. Urinary tract includes the organs that collect and store urine and release it from the body which include: kidneys, ureters, bladder and urethra. (UTIs) are among the most common bacterial infections in humans, both in the community and hospital settings and have been reported in all age groups in both sexes [5]. It is a serious health problem affecting millions of people each year and is the leading cause of Gram-negative bacteria. UTIs are also the leading cause of morbidity and health care expenditures in persons of all ages. The accurance of *Pseudomonas aeruginosa* (5.3%) in UTI patient but it is multi drug resistant bacteria and also cause many diseases in infected person. The commonest source of *P. Aeruginosa* causing hospital acquired UTIs is the environment where it is widely distributed in soil, water, sewage, plants and animal surfaces. The ability of *P. Aeruginosa* to colonize and thrive in diverse environments is reflected by its relatively large genome size and genetic complexity. Large number of genes encoding outer membrane proteins, resistance – nodulation – cell division, efflux systems and multiple complex chemotaxis systems may contribute to its pathogenicity. The crucial criterion which remains to be evaluated is the potential *in vivo* pathogenicity of *P. Aeruginosa* from environmental sources since it is an opportunistic pathogen. India unquestionably occupies the top position in the use of herbal drugs. It is one of the foremost countries exporting plant drugs and their derivatives [6]. Nature has been a source of medicinal agents for thousands of years and since the beginning of mankind. The application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession [7]. Medicinal plants are cheap and renewable sources of pharmacologically-active substances and are known to produce certain chemicals that are naturally toxic to bacteria [8].

EXPERIMENTAL SECTION

Collection of samples.
Urine samples were collected from patient, infected with UTI from civil hospital of Paonta Sahib, Himachal Pradesh. The samples were collected aseptically in sterile tubes.

Enrichment of samples.
The samples were inoculated in Nutrient Broth for 24 hrs.for enrichment. 1ml of samples were added to 9 ml of Nutrient Broth for sufficient enrichment and incubated at 37^0 C.

Recovery of isolates of *pseudomonas aeruginosa.*
The enriched urine samples were streaked on selective media pseudomonas isolation base agar with the help of calibrated loop and incubated at 37^0C for 24 hrs. for recovery of isolates of *pseudomonas aeruginosa.*

Gram staining and colony characterization.
Isolated colony was identified by colony character and gram staining.

Biochemical tests.
The biochemical tests carried out were motility, Urease, citrate utilization, Catalase, Indole, hydrogen sulphide production, methyl red Voges-Proskauer (MRVP) sugar fermentation tests[9].

Antibiotics susceptibility test.
Antibiotics susceptibility was carried out on *pseudomonas aeruginosa positive* isolate using Kirby-Bauerdisc diffusion method. The resistance and sensitivity of isolates toward all the antibiotics were studied by using triplicate plates of MHA. Results were interoperated by measuring the zone of inhibition in mm.

Collection and Extraction of plant materials
The plant materials used were the fruit of *Kigelia africana* and root of *Carica papaya*collected from village area of Dehradun and identified by Botanical Survey of India, Dehradun. For this purpose dried and powdered of fruit and root of plant were extracted with different solvents (ethanol, aqueous) by Soxhlet apparatus. By removing the solvents in Rotary Evaporator (Butchi Type) at 70-80^0 C crude extract was obtained.

Phytochemical Screening of plant extracts
Alkaloid was investigated according to procedure given by [8].
Saponin and Flavonoids: were investigated according to the procedure of [8].
Tannins: Tannins were investigated according to the procedure of [9].
Glycoside: Glycosides were investigated according to the procedure of [10].

Antibacterial activity of medicinal plant.
Antibacterial activity of medicinal plant was determined by agar well diffusion method. The antimicrobial drugs occupy a unique vehicle in the history of medicine. The realization that certain microorganisms are successfully resisting the “wonder drugs” [13].

RESULT AND DISCUSSION

In the present study *Pseudomonas aeruginosa* was isolated and identified by various test like gram staining, colony characterization and biochemical testing and it is resistant to multi antibiotic and results are tabulated in **table 1**. Our isolate was multi drug resistant it was sensitive only to Ofloxacin, ticaracilin. This is similar to the study of [14], in which gram negative bacterial isolates of UTI were multi drug resistant.

Table no 1 Antibiotic Susceptibility test

Antibiotic used	
Ampicillin	Resistant
Amoxylin	Resistant
Ceftizoxime	Resistant
Cefepime	Resistant
Ciphloridine	Sensitive
Ticaracillin	Resistant
Tetracycline	Resistant
Claxacin	Resistant
Erthyomycin	Resistant
Oflaxacin	Sensitive

The results of Phytochemical screening of ethanol and aqueous extracts *C.papaya* revealed the presence of alkaloids, flavonoids, saponins and tannins are tabulated in **Table 2** and Polyphenols and flavonoids are used for the prevention and cure of various diseases [15] which is mainly associated with free radicals. Our study was similar to the study of [16] and the results of

ethanolic and aqueous extracts of *Kigelia africana* revealed the presence of alkaloids, glycosidase, flavonoids, saponins which is similar to the previous study of [17].

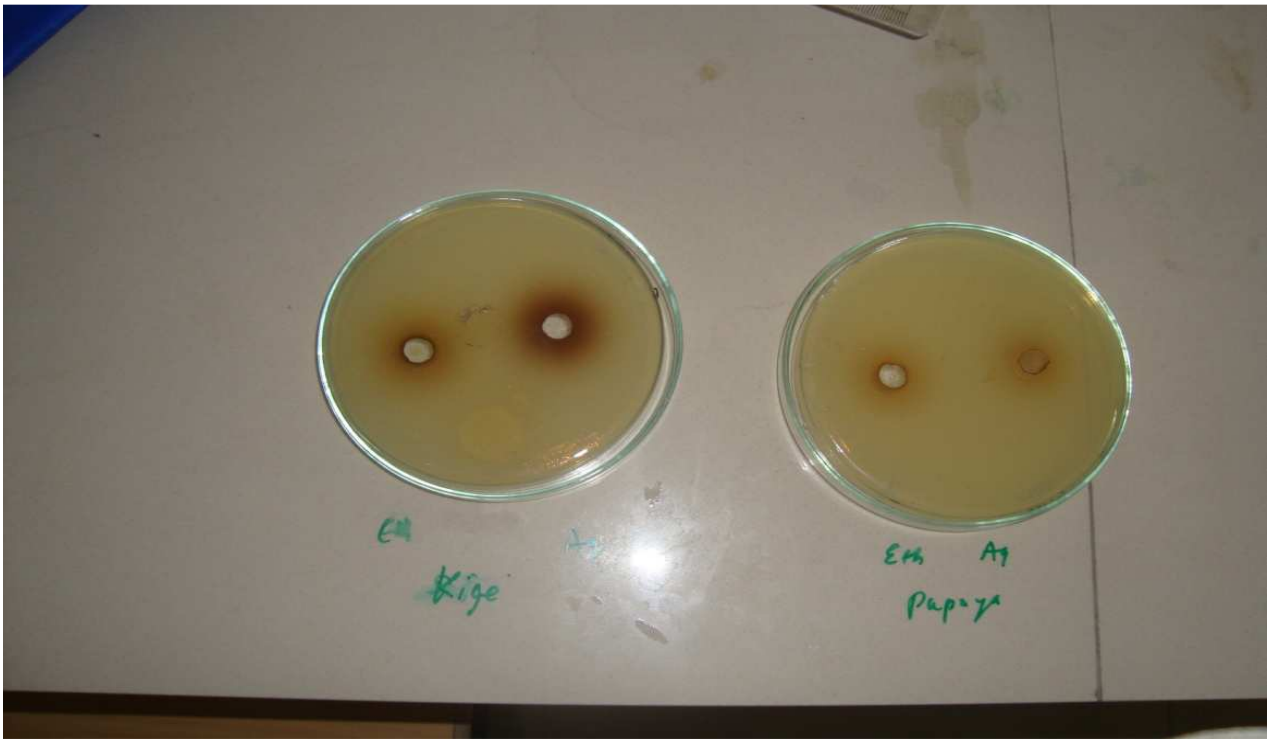
Table 2 Phytochemical screening of plant extracts

Plant extract	Alkaloids	Glycosides	Flavonoids	Saponins	Tannins
Aq. C. papaya	-ve	-ve	-ve	+ve	+ve
Eth. C. papaya	+ve	-ve	+ve	-ve	+ve
Aq. Kigelia	+ve	+ve	+ve	+ve	-ve
Eth. Kigelia	+ve	+ve	+ve	-ve	-ve

Both ethanolic and aqueous extracts of *Carica papaya*root extracts shows antibacterial activity 13 mm and 16 mm respectively against *Pseudomonas aeruginosa* in **Table 3** which are slightly different from the previous study of [16]Yusha 2009 according to them leaf extract shows 7 mm zone. And the ethanolic and aqueous extracts of *Kigelia africana* shows antibacterial activity 20 mm and 17 mm zone which is similar to some extent of the study of [18] according to their study *Kigelia* shows antibacterial activity against many bacteria.

Table 3 Antibacterial activity of plant extracts

Plant extract used	**Zone in diameter (mm)**
Aq. *Carica papaya*	13
Eth.*Carica papaya*	16
Aq.*Kigeliaafricana*	17
Eth.*Kigeliaafricana*	20



CONCLUSION

Both aqueous and Ethanolic extracts of *Kigelia* and *Carica papaya* shows antibacterial activity against *pseudomonas aeruginosa* but *Kigelia* is more effective as compared to *Carica papaya* and ethanolic extract of *Kigelia* shows more activity as compared to aqueous extract of *Kigelia*. From this study we conclude that use of these medicinal plants as antibacterial agent againstUTI infected patient.

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REFERENCES

[1] Stamm WE, Norrby SR. *J Infect Dis*. **2001**, 183,Suppl 1:S1-S4.
[2] Stapleton AE. *Curr Treat Opt Infect Dis*.**2003**, 5, 43-51.

[3] Mandell GL, Bennett JE Dolin R. Principles and practice of infectious diseases. Churchill Livingstone.**2005**, 881-882.
[4] Ebie MY, Kandakai-Olukemi YT, Ayanbadejo J, Tanyigna KB. *Niger. J. Microbiol*. **2001**,15(1), 31-37.
[5] Hooton TM, Winter C, Tiu F, Stamm WE. *JAMA.***1995**, 273, 41-5.
[6] Chulet Rahul, Pradhan Pankaj, Sharma K Sarwan, Jhajharia K Mahesh. *J. Chem. Pharm. Res*., **2010**, 2(5): 476-484
[7] Pranay Jaina and Rishabh Varshney. *J. Chem. Pharm. Res.*, **2011**, 3(3):260-263
[8]Basile, A. Giordano, S Lopez-saez, J A and Cobianchi RC. *Phytochemistry*.**1999**, 52, 1479-1482**.**
[9]Holt JG, Krieg NR, Sneath PH, Stanley JT and Williams ST. Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Baltimore.**1994**, pp. 529-550.
[10] Harborne, J.B. Phytochemical methods, London. Chapman and Hall, Ltd.**1973**, pp. 49-188.
[11] Trease, G.E., Evans, W.C. Pharma cognsy.11th Edn. BrailliarTiridel Can. Macmillan Publishers.**1989**.
[12] Siddiqui, A.A., Ali.Practical Pharmaceutical chemistry.1st ed. CBS Publishers and Distributors, New Delhi.**1997**, pp . 126-131.
[13] Kokila A. Parmar, Anup N. Patel , Sarju N. Prajapati , Rakesh I. Patel. *J. Chem. Pharm. Res*., **2010**, 2(4):324-332
[14] Stickler DJ And Thomas B. *J ClinPathol*. **1980**, 33, 288-296.
[15] Damiki Laloo and Alakh N Sahu. *J. Chem. Pharm. Res*., **2011**, 3(1):277-283
[16] Yusha'u, M., Onuorah, F. C. and Murtala, Y. *Bayero Journal of Pure and Applied Sciences*.**2009**, 2(2): 75 – 78
[17] Azu, O. O., Duru, F. I. O. Osinubi, A. A, Noronha, C. C., Elesha, S. O. and Okanlawon A.O. *African Journal of Biotechnology*.**2010**, 9, 1374-1381.
[18] Jeyachandran, R. and A. Mahesh. *Res. J. Microbiol*. **2007**, 2, 645-649.