INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

Antibacterial Potential of Phytochemicals against Urinary Tract Infectious Bacteria

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Abstract:
Urinary Tract Infections represent one of the most common diseases encountered in medical practices. The clinical significance of the disease is due to its high mortality rate, malignant and chronic hypertensions and chronic renal failures. The present study involves the isolation of bacterial strains by catheterization and identification based on their morphological characterization and biochemical analysis. The disc diffusion assay was performed for a comparative analysis to identify the most suitable plant extract and its active compound that could be used in curing ailments of Urinary Tract Infections. The plant extracts of Eupatorium odoratum L.,Azadirachta indica L., Ocimum sanctum L. Coleus aromaticusL., Aloe barbadensis L., Rotula aquatica L, were analyzed for their antibacterial activity against the isolates. Further, the active compounds, lignins and proteins were isolated from the effective extracts of Eupatorium odoratum L. and Rotula aquatica L. and tested for their antibacterial activity which could be used in the formulation of various medicines. Four different bacterial isolates obtained were identified asE. coli,Klebsiella pneumoniae,Pseudomonas aeruginosa and Staphylococcus aureus. The most effective plant extracts were that of Eupatorium odoratum L. and Rotula aquatica L. The protein extract of Eupatorium odoratum L. and Rotula aquatia L.proved to be effective constituent as antibacterial agent.The excessive use of antibiotics paves way for secondary ailments and resistance between different bacterial species, hence there is a rapid replacement of chemical antibiotics with natural remedies which is highly demanded for their non toxicity and lesser side effects and better cure.

Keywords: *Urinary Tract Infections, Antibacterial, Active compounds*

1. Introduction

Urinary Tract Infections represent one of the most common diseases encountered in medical practices and encompasses a broad range of clinical fields that are associated with a common finding of positive urine cultures (Hosseini *et al.*, 2012). There are an estimated 150 million Urinary Tract Infections per year worldwide (Kunin,1994). The disease is prevalent in one of the parts of the Urinary Tract such as Kidneys, Ureters, Bladder and the Urethra. Urethritis, Cystitis, Acute Pyelonephritis, Prostatitis and intra-renal and Perinephric abscesses are thecommon types of urinary tract infections. (Nahar *et al*.,2014). The clinical significance of the disease is due to its high mortality rate, malignant and chronic hypertensions and chronic renal failures followed by chronic Pyelonephritis (Sharifian *et al*.,2006)

The infection varies in patients with different gender, age and presence of associated genitourinary abnormalities (Lee and Neild, 2007).

Catheter associated urinary tract infections are caused by a variety of pathogens including*Escherichia coli, Klebsiella, Proteus, Enterococcus, Pseudomonas, Enterobacter, Serratia* and *Candida*. Many of these microorganisms are part of the patient's endogenous bowel flora, but they can also be acquired by cross contamination from other patients or hospital personnel or by exposure to contaminated solutions or nonsterile equipment (Gleckman, 1992).*E. coli* is the most common organism causing both community as well as hospital acquired urinary tract infections. Those infections acquired in hospital, following instrumentation, are more often caused by *Pseudomonas.*

Medicinal plants have been known for millennia and are highly esteemed all over the world as a rich source of therapeutic agents for the prevention of various ailments. Today large number of population suffers from kidney stones, gallstones and urinary calculi.

Medicinal plants are frequently used as raw materials for extraction of active ingredients which is used in the synthesis of different drugs. Curing ailments by natural cure is highly effective because the ingredients of plants all interact simultaneously, so their uses

can complement or damage others or neutralize their possible negative effects. The present study was undertaken to analyze the bacteria present in urine samples and their susceptibility patterns to selective plant extracts.

2. Materials and Methods

- Collection of Urine samples: The collection of Urine samples was made by Catheterization from patients at Lisie Hospital, Ernakulam.
- Isolation of bacteria from the samples: Isolation of bacteria from the sample was done by streak plate method. The different strains of bacteria were grown on nutrient agar medium.
- Identification of bacterial strains: Preliminary identification of the bacteria was done on the basis of colony characteristics. The identification of the isolates was further confirmed by biochemical analysis.

2.1. Antibacterial Analysis of Plant Extracts

The antibacterial analysis of plant extracts was done using plant extracts, reported to have potential for curing Urinary Tract Infections and is a chief ingredient of ayurvedic formulations. The plants having potential for curing urinary tract infections that were used and the parts that were selected for the study included the leaves of A*zadirachta indica* L.*, Eupatorium odoratum* L. *Ocimum sanctum* L., *Coleus aromaticus* L.*,* and *Aloe barbadensis* L.and the finely ground stem of *Rotula aquatica* L.

2.2. Preparation of the Plant Extracts

The plant extracts were prepared by first washing the plant parts with distilled water 2-3 times for removing the dirt and other soil contaminants, and 20gm of the plant part was accurately weighed and ground in pestle and mortar using 50 ml of 100% acetone.
10 concentrations were selected by serial dilution method for disc plate method from 100% to 10%.
The plant extracts which proved ideal were further subjected to isolation of lignins and proteins which were tested for antibacterial action by disc diffusion method.

2.3. Isolation of Proteins from Plant Tissue

Isolation of Proteins from plant tissue was done by Protein acetylation (Sensi *et al*., 2003).

2.4. Isolation of Lignins from Plant Tissue

Isolation of Lignin was done by Lignin acetylation (Doherty *et al*., 2010).

2.5. Testing Of Isolated Proteins and Lignins for Antibacterial Activity

The isolated proteins and lignins were tested for antibacterial activity using disc diffusion method. The plant extracts of *Eupatorium odoratum* L. and *Rotula aquatica* L. were used for the purpose. The bacterial strains that exhibited the highest inhibition zone were used to test for the effect of proteins and lignins. The petriplates containing the Mueller Hinton agar medium was swabbed with the corresponding bacterial strain and was seeded with the paper discs at equal distance. Using sterile micropippete 0.5ml of the protein and lignin extracts were introduced onto the paper discs. The petriplates were incubated at 37◦C for 24 hours. The clear zone was observed and measured using a scale.

3. Results and Observation

3.1. Results

3.1.1. Isolation Characterization, Biochemical Analysis and Identification of Bacterial Strains Present in Urine

Four strains of bacteria were isolated from the sample. The strain S1LH gave mucous colonies on culturing and was positive for Mannitol motility test, Triple sugar iron agar test and Indole test and hence identified as *Escherichia coli*. The strain S2LH gave normal flat colonies and gave positive results for Mannitol motility test and Citrate test and hence identified as *Klebsiella pneumoniae*. The strain S3LH produced green pigmented colonies and was positive only for Citrate test hence the strain was identified as *Pseudomonas aeruginosa*. S4LH strain had small rounded colonies and was positive only for Coagulate test and hence identified as *Staphylococcus aureus* (Table 1)

STRAIN	COLONY CHARACTERISTICS	MMT	TSI	INDOLE TEST	CITRATE TEST	COAGUALSE TEST	IDENTIFICATION OF BACTERIAL STRAIN
S1LH	Mucoid colonies were observed	+	+	+			*Escherichia coli*
S2LH	Normal flat colonies were observed		+		+		*Klebsiella pneumoniae*
S3LH	Green pigmented colonies observed				+		*Pseudomonas aeruginosa*
S4LH	Small rounded colonies were observed					+	*Staphylococcus aureus*

Table 1: Isolation Characterization, Biochemical analysis and Identification of Bacterial strains present in Urine

3.1.2. Antibiotic Sensitivity Test Using Plant Extracts for Curing Urinary Tract Infections

According to the Category of Plant extracts the most effective of the plant extracts were *Eupatorium odoratum* L. and *Rotula aquatica* L. extracts, effective against all bacterial strains at least in one concentration (Table 2).

PLANT EXTRACTS	*Escherichia coli*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
	80%	20%	80%	20%	80%	20%	80%	20%
	Zone size in mm							
Aloe barbadensis	-	-	-	-	-	-	-	-
Azadirachta indica	23	15	-	14	14	12	-	-
Coleus aromaticus	-	-	-	12	-	-	-	-
Eupatorium odoratum	23	16	25	17	-	17	-	10
Ocimum sanctum	23	13	12	10	-	-	-	-
Rotula aquatica	20	17	16	15	21	22	12	-

Table 2: Antibiotic Sensitivity test using plant extracts having potential for curing Urinary tract infections

3.1.3. Antibacterial Effect of Proteins and Lignins Isolated from Plant Extracts

The protein extracts of *Eupatorium odoratum*L. and *Rotula aquatica* L. was very effective against *E. coli* strain exhibiting maximum inhibition zone when compared to Lignin extracts (Table :3).

BACTERIAL STRAINS	*Eupatorium odoratum* Protein extracts		*Eupatorium odoratum* Lignin extracts		*Rotula aquatica* Protein extracts		*Rotula aquatica* Lignin extracts	
	Zone size in mm							
Eschertchia colt	13	12	13	10	17	14	15	10
Klebsiella pneumoniae	13	10	12	10	12	10	13	10
Pseudomonas aeruginosa	17	15	11	10	13	11	12	10
Staphylococcus aureus	Resistant	Resistant	10	Resistant	Resistant	Resistant	Resistant	Resistant

Table 3: Antibacterial effect of proteins and lignins isolated from plant extracts using disc plate method

4. Discussion

Urinary Tract Infections remains the most common bacterial infection in human population and is one of the most frequently occurring nosocomial infectionsRonald *et al.,* (2001). The present study investigated the existence of bacterial strain present in the Urinary tract as a result of Catheterization. The samples obtained indicated the presence of four different bacterial strains which were subjected to primary investigation by culturing on nutrient agar and studying the morphological nature of the colonies so obtained, after which was subjected to disc diffusion method using plant extracts as to identify the most suitable antibacterial agent.

Plant extractshave been widely used as antibacterial agents and was found to be chief ingredient in production of various medicinal formulations in the present scenario, especially for urinary tract infections. In the present study the plant extracts of *Eupatorium odoratum* L. and *Rotula aquatica* L. were found to be very effective against all bacterial strains. A study on antibacterial effect of *Eupatorium adenophorum* against bacterial isolates was studied by Bhattarai and Shrestha (2009). *Eupatorium adenophorum* L. extract showed selective effect towards the bacterial strains of*Proteus* spp., Salmonella spp., Staphylococcus spp., Bacillus*subtilis*, B.*thurengiensis*, B. *cereus*, Enterobacter *aerogenes*, *Salmonella paratyphi*, Staplococcus *aureus and Proteus mirabilis.*

The present study also gave similar results with the *Eupatorium odoratum* L. extract giving the maximum inhibition zones with bacterial strains of *Escherichia coli, Klebsiella pneumoniae, Pseudomonasaeruginosa*and Staphylococcus *aureus.*

The present study also analyzed the antibacterial action of the specific active compounds of lignins and proteins by the isolation of compounds from the effective plant extracts. The Protein extracts of *Eupatorium odoratum* L. proved to be the most effective as per the current study.

In the present scenario urinary tract infections are highly prevalent due to the lack of drinking pure water and also for finding a substitute for drinking water. The infections may arise as a result of catheterization or as a secondary infection.

The use of plant extracts and phytochemicals, both with known antibacterial properties,can be of great significance in therapeutic treatments. Intensive care physicians consider antibiotic resistant bacteria a significant problem in the treatment of diseases. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds. Medicinal plant extracts, therefore offer considerable potential for the development of new agents effective against infections currently difficult to treat.The finding that the protein extract in *Eupatorium odoratum* L. and *Rotula aquatica* L. have potential antibacterial activity promises the development of new therapeutic measures to cure urinary infections without much side effects.

5. Summary and Conclusion

Urinary Tract Infections (UTI) are most common form of bacterial infections, affecting people throughout their life span. The pathogenesis of complicated and uncomplicated UTI is complex and influenced by many host biological behavioral factors and properties of infecting uropathogens.

The present study envisages to isolate, identify bacteria in urine samples, to analyze the plant extracts for their antibacterial property to be used against urinary infections. Urinary samples were obtained by catheterization. Four strains of bacteria were isolated and identified by culture and preliminary identification was done on the basis of characteristics of the colony. Leading etiological agents of UTI's identified were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* which were confirmed using biochemical analysis.

The plant extracts of *Eupatorium odoratum* L. and *Rotula aquatica* L. were the only extracts that were effective against bacterial strains of *Escherichia coli*, *Klebsiellapneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. The effective plants extracts were further subjected to isolation of lignins and proteins and analyzed for antibacterial action. The protein extract of *Eupatorium odoratum* L.proved ideal as an antibacterial agent.

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus they could be used in the treatment of infectious diseases caused by resistant microbes.

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