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Bioactive Studies of *Mangifera indica* aganist Bacteria Isolated from Urine Samples

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Abstract: Urinary tract infections are the second most common type of infection in the world. It is usually caused by bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebisella pneumoniae*, *Streptococcus pyogens* etc. Bacteriuria, Pyrexia, Uretheritis, Pyuria, Haematuria are the urinary tract infection, commonly caused by bacteria. Seed kernel of *Mangifera indica* which has antibacterial, antidiarrhoeal, antioxidant and antiviral activity against the uropathogens isolated from clinical samples. Urine samples were collected from government hospital at Srirangam. The samples were subjected to macroscopic, microscopic, and culturing by selective and differential media and biochemical test to identify the pathogens. Seed kernels of *M. indica* were extracted by using water and ethanol. Antibacterial activity of *M. indica* aqueous and ethanol was studied by disc diffusion method, which shows best activity against the clinical isolates at 800 µg concentration (19mm). *St. aureus* is strongly inhibited by the extract. MIC was performed by using agar dilution method. It was found to be between 300mg/ml to 750mg/ml. Phytochemical studies revealed that the presence of Tannin and Steroid. If we develop a good drug from this plant it may be better for human health.

Key words: *Mangifera indica,* seed kernels, *Staphylococcus aureus* and urinary tract infections

INTRODUCTION

Urinary tract infections are serious health problem affecting millions of people each year. Urinary tract infections account for about 8.3 million doctor visit each year. One woman in five develops a UTI during her lifetime. *Escherichia coli, Klbesiella, Streptococcus pyogens, St. faecalis, Pseudomonas vulgaris, P. faecalis* are the bacteria responsible for urinary tract infection. Commonly used antibiotics for the treatment of urinary tract infection are Clindamycin, Vancomycin, Bacitracin, Ampicillin, Chloramphenicol, and Erythromycin. These antibiotics may reduce the burden but it has its own side effects. To overcome the problems associated with antibiotic treatment, people turned traditional medicine like Ayurveda, Siddha, unani, Homeopathy and herbal medicines. About 80% of world populations rely on herbal medicine for primary health care (WHO/WPRO, 1998). Thousands of medicinal plants available locally are used to treat urinary tract infection. Some of the plants leaves of *Punica grantaum*, seed kernel *Mangifera indica*, whole plant of *Tridax procumbans*, whole plant of *Aerva lannata* were already used to control UTI. Based on various usages in the present study we have chosen seed kernel of *M. indica* to screen antibacterial activity, antidiarrhoeal activity and its phytochemistry.

MATERIALS AND METHODS

Urine samples were collected from clinically diagnosed urinary infectious patients admitted in Government hospital, at Srirangam, Tamil Nadu, India. Samples were collected for three months from (January-March, 2007).

Urine samples were collected from clinically diagnosed cases of Urinary tract infection. Seed kernel of *M. indica* was the plant materials chosen for anti-bacterial activity studies. *E. coli, S. aureus* and *St. pyogens were* isolated from the urinary tract infectious patients were used as test organism.

Both broad spectrum and narrow spectrum antibiotics were used to assess sensitivity pattern of the clinical isolates i.e., *E. coli*, *S. aureus* and *St. pyogens.* Antibiotics like Azithromycin (15µg), Carbenicillin (100µg), Cefuroxime (10µg), Chloramphenicol (30µg), Doxycycline hydrochloride (30µg), Minocycline (30µg), Nalidixicacid (30µg), Rifamycin (5µg), Vancomycin (30µg), Clarithromycin (15µg), Trimethoprim (5µg), Spectinomycin (100µg), Amoxyclave (30µg), and tetracycline (30µg), Co-trimoxazole, Clinadamycin, Erythromycin and Bacitracin, were used to see the sensitivity pattern of enteric pathogens.

Source of Plant Material: Good quality Seed kernel of *M .indica* was collected locally and identified.

Urine collection: Urine samples were collected in a wide mouthed container (Hi-media) from clinically diagnosed patients. After collection of samples, the containers were closed tightly to avoid any leakage during transportation, (Koneman *et al.,* 1994). Various types of processing were done to find out the correct etiological agent of the disease. Processing varies in accordance with the aim of the study and the pathogens looked for.

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E. coli is one of the gram negative bacteria. It belongs to the family Enterobacteriaceae. Enrichment, differential and selective media were used to isolate *E. coli*. *Staphylococcus* and *Streptococcus* is the gram-positive organism. Differential and selective media were used to isolate these organisms.

A loop full of urine was taken and inoculated on Macconkey agar and Blood agar with antibiotics disc for differentiation of gram positive organism and was incubated at 37 ºC for 24 h under aerobic condition. Pink color colony and hemolytic colonies was selected from Macconkey agar and Blood agar and it was subjected to sub culturing for further screening procedure. All of them were incubated aerobically at 37 ºC for 24 h and were looked for specific colony morphology, which would confirm the isolation of *E. coli, S. aureus and St. pyogens*. *The* growth was observed in selective and differential medium and the results were tabulated.

E. coli, S. aureus and St. pyogens was identified by making use of biochemical tests in addition to its growth characters on nutrient agar and microscopic analysis.

Selected colonies from selective and differential media were subjected to microscopy, microscopy and biochemical tests for identification.

Microscopic observations like size, shape and motility reveal the availability of different morphological characters among microorganisms. Simple staining, gram staining and hanging drop methods were done to look for their shape, grams nature and motility of the isolate respectively (Henry, 1994).

Gram staining was performed to look for the grams nature of the isolate. A purple coloured cell retains grams crystal violet and was called gram positive bacterium. Pink coloured cells lost primary stain and picked up safranin color and were called as gram negative bacterium.

Bacteria were motile by their flagella. The number and location of which vary among different species. Motility can be observed directly by hanging drop technique that is by placing a drop of culture on a microscopic slide and looked under microscope by keeping them inverted.

Physiological and metabolic characteristics of the microorganisms were assessed through biochemical tests. These characteristics are very useful because they are directly related to the nature and activity of microbial enzymes and transport proteins. Analysis of these characteristics provides an indirect comparison of microbial genomes. The following tests were used to characterize microbial enzymes and proteins.

Indole test, Methyl red test (MR) Voges Proskauer test (VP), Citrate utilization test (C), Urease production test (U), Nitrate reduction test (N), Cytochrome oxidase activity , Catalase test were tested by adopting the method of Koneman *et al.* (1998)

Inhibition by Furazolidone: It was performed by preparing a suspension of the test organism in distilled water or broth equivalent to the test organism in suspension and spread onto one half of a blood agar plate. A 100 mg Furazolidone disc is placed aseptically in the center of the inoculated area. The plate is incubated at 35ºC for 18-24 h and observed for zone formation. *Staphylococci* are inhibited by Furazolidone and shows zone of 15 mm. The coagulase negative *Staphylococci* are resistant to Furazolidone.

Resistant to Bacitracin: The Bacitracin disk was used for the presumptive identification of group A, Beta-hemolytic streptococci on Muller-Hinton Agar or Blood agar plate. After incubation, *Staphylococcus* was resistant to Bacitracin.

Slide coagulase test: The tests were performed by placing two drops of sterile water or saline on a slide. The well isolated colonies of organism are emulsified in the liquid within each of the circle. A drop of coagulase plasma is added to one of the suspension and mixed with a wooden applicator stick; similarly, a drop of water or saline is added and mixed in the other suspension as control. The suspension is then observed for agglutination.

DNase test: *St.aureus* strain procedure weak or equivocal tube coagulase reaction, which reaction may be helpful to perform other test. *S. aureus* produce DNase and thermostable endonuclease. These hydrolyze nucleic acid change the colour formation of metachromatic dye toluidine blue O into pink. After incubation period for 24 h at 37º C it indicates the hydrolysis of the DNA.

Hydrolysis of L-pyrrolindonyl B-naphthylamide (PYR): PYR hydrolysis is a presumptive test for both groups A and D *Enterococcal* and *Streptococci*. This test is highly sensitive. It replaces the Bacitracin test and the salt tolerance test for group A, *Streptococci*.

Bile-Esculin test: To hydrolyze esculin in the presence of 40% bile is used for the presumptive identification of group D *Streptococci* and *Enterococcus* sp. This test is generally performed on an agar slant or in a plate that contain the bile-esculin medium. Any blackening of the agar in the plate indicates a positive result.

Salt Tolerance Test (6.5% Nacl broth): The salt tolerance test was based on the ability of an organism to grow in 6.5% NaCl, separates the *Streptococcal* sp. The organism to be identified is inoculated into an infusion based agar or broth containing 6.5% NaCl. After incubation, the medium is observed for the growth.

Susceptibility to Ethyl hydrocupreine hydrochloride (optochin): To perform the test, a few colonies are sub cultured to a Blood agar plate and are streaked as lawn. An optochin disc is placed on the inoculum and the plate is incubated at 35ºC in 5% CO_2. A zone of 14 mm or greater around the 6mm indicates the susceptibility to optochin.

Bile Solubility test: The test can be performed on a broth or saline suspension of the organism or directly on a plate. In the tube test, clearing of the 10% deoxycholate suspension after the inoculation of unknown organism and incubation for 3 h indicates lysis of the bacterial cells. For the plate test, a drop of 2% sodium deoxycholate is placed directly on a few colonies of the organism and incubated at 35ºC without inverting for 30 min. The colonies will lyses and disappears leaving only the area of haemolysis.

RESULTS

We have collected 25 samples from government hospital at Srirangam. All these samples were collected from clinically diagnostic cases. Samples were categories based on sex. About 68% of our samples were from female and it is double times higher than males (32%) (Table 1).

All the clinical samples were subjected to microbiological examination. In the present study bacterial etiology was noted 18 samples out of 25% (72%) (Table 2).

Bacteria are the group of microorganism that belongs to the group prokaryotes that multiplied with in the Urinary Tract and produced severe Urinary Tract Infection. Uropathogens are responsible for majority of human infection. Bacteria are the major flora that causes severe Urinary Tract infection then other microbial groups. Culture media like Blood Agar, Macconkey Agar are primarily used for the recovery of uropathogens. Totally 10 *E. coli*, uropathogens are isolated from the clinical samples (55%) followed by *S. aureus* (5 numbers and 28%) *St. pyogens* (3 numbers and 17%).Various biochemical test were used for the diagnosis of uropathogens (Table 3).

Antibiotics are medicines that fight against infections caused by bacteria. Because antibiotics are used a lot something used in appropriately, antibiotic resistant is becoming a common problem in many parts of the world.

Table 1: Samples collection details and categories based on sex

Total No. of samples	Male	Female
25	8	17

Table 3: Incidence of bacterial etiology selected for antibacterial screening

S No	Isolated organisms	Number of isolates
1	*E.coli*	10
2	*Staphylococcus aureus*	5
3	*Streptococcus pyogens*	3

Table 2: Identification of Bacteria from Urinary tract isolates

Media	*E. Coli*	*Streptococcus Pyogens*	*Staphylococcus aureus*
EMB	Metallic Sheen Colour	Non - Metallic Sheen Colour	Non - Metallic Sheen Colour
XLD	Yellow colour	colourless	colourless
Macconkey	LF	NLF	NLF
Blood Agar	-	β- Hemolysis Van – R	β- Hemolysis Bac – Sen
Baired Parker Agar	-	Black Colour Vanc – R	-

NM	-Non Motile
M	-Motile
NP	-Not Performed
+	-Positive
-	-Negative
Van - R	-Vancomycin Resistant
Bac – Sen	-Bacitracin Sensitive
Streptococcus Pyogens	-US1, US14, US15,
Staphylococcus aureus	- US3, US7, US8, US9, US16
E. Coli	- US2, US4, US5, US6, US10, US11,US12, US13, US17, US18
A/A	-Acid / Acid

Table 2: (Continue)

S.No	Test	US1	US2	US3	US4	US5	US6	US7	US8	US9	US10	US11	US12	US13	US14	US15	US16	US17	US18
1	Gram staining	+	-	+	-	-	-	+	+	+	-	-	-	-	+	+	+	-	-
2	Shape	Cocci	Rod	Cocci	Rod	Rod	Rod	Cocci	Cocci	Cocci	Rod	Rod	Rod	Rod	Cocci	Cocci	cocci	Rod	Rod
3	Motility	N/M	M	N/M	M	M	M	N/M	N/M	N/M	M	M	M	M	N/M	N/M	N/M	M	M
4	Indole test	-	+	-	+	+	+	-	-	-	+	+	+	+	-	-	-	+	+
5	Methyl red test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Vogespros kaur test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Citrate utilization test	NP	-	NP	-	-	-	NP	NP	NP	-	-	-	-	NP	NP	NP	-	-
8	Urease test	NP	-	NP	-	-	-	NP	NP	NP	-	-	-	-	NP	NP	NP	-	-
9	TSI test	NP	A/A	NP	A/A	A/A	A/A	NP	NP	NP	A/A	A/A	A/A	A/A	NP	NP	NP	A/A	A/A
	H2S	NP	-	NP	-	-	-	NP	NP	NP	-	-	-	-	NP	NP	NP	-	-
	Gas	NP	+	NP	+	+	+	NP	NP	NP	+	+	+	+	NP	NP	NP	+	+
10	Nitrate reduction test	NP	+	NP	+	+	+	NP	NP	NP	+	+	+	+	NP	NP	NP	+	+
11	Catalase tests	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
12	Oxidase test	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
	Carbohydrate test																		
13	Glucose	NP	+	NP	+	+	+	NP	NP	NP	+	+	+	+	NP	NP	NP	+	+
14	Maltose	NP	+	NP	+	+	+	NP	NP	NP	+	+	+	+	NP	NP	NP	+	+
15	Sucrose	NP	+	NP	+	+	+	NP	NP	NP	+	+	+	+	NP	NP	NP	+	+

Table 4: Antibiotic Sensitivity Assay Patterns of Clinical Isolates

S.No	Antibiotics	*Ecoli*		*Streptococcus pyogens*		*Staphylococcus aureus*	
		S	R	S	R	S	R
1.	Deoxycline hydrochloride	50%	50%	80%	20%	33%	67%
2.	Rifampicin	40%	60%	60%	40%	67%	33%
3.	Azithromycin	30%	70%	40%	60%	33%	67%
4.	Spectinomycin	20%	80%	20%	80%	67%	33%
5.	Trimethoprin	70%	30%	40%	60%	67%	33%
6.	Cefaroxine	60%	40%	60%	40%	33%	67%
7.	Minocyclin	70%	30%	80%	20%	67%	33%
8.	Clarithromicin	20%	80%	60%	40%	33%	67%
9.	Vancomycin	50%	50%	40%	60%	33%	67%
10.	Carbenicillin	30%	70%	20%	80%	67%	33%
11.	Erythromycin	40%	60%	80%	20%	33%	67%
12.	Chloromphenicol	70%	30%	60%	40%	67%	33%
13.	Trimoxazole	50%	50%	40%	60%	33%	67%
14.	Clindamycin	20%	80%	80%	20%	67%	33%
15.	Amoxyclave	60%	40%	60%	40%	33%	67%
16.	Tertracuicline	70%	30%	40%	60%	33%	67%
17.	Nalidicin	60%	40%	20%	80%	67%	33%
18	Bacitracin	60%	40%	80%	20%	33%	67%

Table 5: Antibacterial activity of *M. indica* against enteric pathogens

S.No	Extracts	Zone of inhibition *E. coli*.(800µg/disc)(mm)	Zone of inhibition *Staphylococcus aureus*(800µg/disc)(mm)	Zone of inhibition *Streptococcus pyogens* (800µg/disc)(mm)
1	Aqueous extract	13	19	11
2	Ethanol extract	8	19	12
3	Positive control	14	22	27
4	Negative control	Nil	Nil	Nil

Table 6: Phytochemistry of *M. indica* extracts

S No	Tests	Aqueous extract	Ethanol extract
1	Carbohydrate	+	+
2	Protein	-	-
3	Alkaloids	+	+
4	Glycosides	-	-
5	Terpenoids	-	-
6	Flavanoids	-	-
7	Tanins	+	+
8	Saponins	+	+
9	Steroids	-	-
10	Starch	+	+

+ - Positive, - - Negative

To know the antibiotic resistant pattern of our isolates we conducted antibiotics assay by making the use of Disk Diffusion method.

About 18 commonly used antibiotics are subjected for the study. Out current report showed that maximum number of isolates were sensitive to new generation antibiotics like carbenzilin, Chloramphenicol, Tetracycline etc. About 75% of *E.coli,* were resistant to amzoxyclave and 80% of *S. aureus* to carbenzilin and more than 60% of *St. pyogens* to Deoxycycline, Rifampicin, carbenzilin, Erythromycin, etc. (Table 4).

M. indica is a medicinal tree commonly called as ma in Tamil and mango in English. All parts of this tree are commonly used in traditional system of medicine. Seed kernel of *M. indica* is selected for the study due to its common medicinal usage. The seed kernel is collected from local market of Srirangam and processed to extract its content by making use of water and ethanol. Both of these extract showed inhibitory activity against all the pathogens tested. Ethanol extract showed better activity then water extract (Table 5).

MIC of *M.indica* extract was studied by using Agar Dilution Method, Which also shows inhibitory activity against all the pathogens. A phytochemical constituent are the principle source of the medicinal plant and is responsible for various biological activities. Tannis are the major component found in both the extract of *M. indica* (Table 6).

DISCUSSION

Urinary Tract Infection are not considered as outbreak diseases but it security is high in human. In general community acquired Urinary Tract Infection occurs mostly in women. Bladder infections are 14 times more common in females than males. Uropathogens is easily entered into female Urinary Tract and cause severe life threading info (Annonymous, 2001).

Our results also revealed the same about 68% of samples were collected from female cases which is two times higher than male. This may be due to shorter urethra. Hormones and chemical barriers present in females urethra.

Randrianirina (2007) also reported that higher incidence of female Urinary Tract Infection (75%) than in male (25%).Multiple factors have probably lead to the emergence and spread of Urinary Tract Infection. Majority of Urinary Tract Infection are caused by uropathogenic bacteria about 72% of Urinary Tract Infection are due to bacteria is noted in the present study. This was also supported by David *et al.* (2005). He reported that common Urinary Tract Infection is caused by bacteria. It means presence of bacteria in urine. Among fact *E. coli* shows higher incidence (Elmanana *et al.,*

2006) isolated 42% of *E. coli* followed by minimum number of other pathogens.

Ronald (2003) reported that Urinary Tract Infection associated with microbial etiology is reasonably consistence. *E. coli*, uropathogens 80% followed by *Klebsiella, Enterobacter, Staphylococcus, and Proteus* etc. is isolated from the urinary tract infection.

Our present study also revealed the presence of 55% of *E. coli* followed by 28% *S. aureus* and 17% of *St. pyogens*. Ronald (2003) indicated that about 10 – 15% of Urinary Tract Infection is due to *staphylococcus*.

Kiffer *et al.,* (2007) isolated 13% of gram positive cocci from the Urinary Tract infected individual from urban areas. We also conducted the study from urban based community environment.

Antibiotic resistance is a major problem of clinical peoples. It is an evaluation process of microorganisms. Majority of Uropathogens developed resistant against second and third generation cephaloporins and other commonly used drugs. We have used 18 antibiotics in the present study. Out of large number of isolates are resistant to multiple drugs and these microorganisms are considered as multi drug resistant pathogens. Resistant to antimicrobial was extremely allomining. Lee *et al.* (2007) showed that *E. coli* resistant to amoxicillin was reached 97.9% to piperacillin 78.3% to Doxycycline, 90% to sulfa-methoxazole 63.9% and to cefaclor 42%.

Over 20,000 practioners of Indian system of medicine in the oral and codified stream uses medicinal plants in preventive, promotive and curative application in Tamil Nadu. *M. indica* and its parts used to cure various purposes. The seed of *M. indica* is reported in traditional medicine as a cure for vomiting, dysentery and burning. Paste is made from mango seed (kernel), honey and camphor and applied over vagina in order to make the vagina contracted and firm (Sharma, 1996).

Seed kernel of aqueous and ethanolic extract inhibited the growth of *S .aureus, P.vulganis* (Sairam *et al.,* 2003). Our aqueous and ethanol extract showed good antibacterial activity, against *E. coli, S. aureus* and *St. pyogens*. Both these extract tract showed best activity against S. *aureus* at 800 µg concentration (19 mm). This antibacterial activity of *M. indica* may be due to specific phytochemical components.

Phytochemical compounds are the key factor to perform biological activities like anti bacterial activity, antifungal, antiprotozoan, antioxidant etc. Tannins are responsible for phytochemical activity. Dried mango seed contain 15% tannin served as astringent in cases of diarrhea, dysentery, uretheritis etc. Toxic components are not detected in seed kernel.

They are the safe source of antioxidant. We also estimated tannins from aqueous ethanolic extract of seed kernel of *M. ind*ica. Saponins, alkaloids starch are also present in these extract.

Medicinal plants are the chief source of medicine and are used for the treatment of varies diseases. Traditionally peoples are using these on regular basis but scientific community not able to accepting the concept. Scientific evidence is needed for the purpose using these plants. The present results also provide evidence for medicinal use of our traditional knowledge.

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