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Antibacterial Potential of Selected Antiurolithiatic Plants on Bacterial Pathogens Isolated from Urinary Tract Infection

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A B S T R A C T

The incidence of Urinary Tract Infection (UTI) was analysed among the patients of Kanyakumari Government Medical College and Hospital, Asaripallam, Kanyakumari district to identify the common bacteria causing UTI and to check antibacterial potential of selected plant extracts against the pathogens. The results showed that 28% of samples yielded significant bacteriuria. Among the infected persons, females were more and percentage of infection was higher in the age group above 55 followed by 35-55 in both sexes. *E.coli* has been found to be the most common UTI bacteria followed by *Klebsiella*. Four bacterial strains i.e. *E.coli*, *Klebsiella*, *Pseudomonas* and *Proteus* were isolated from UTI samples and antibacterial activity of aqueous and methanol extracts of *Aerva lanata* and *Scoparia dulcis* were carried out by disc diffusion method. The highest zone of inhibition was observed in the extracts of *Aerva* root for *E.coli* and *Klebsiella* where as in *Pseudomonas* it was in the fruit extracts of *Scoparia*. *Proteus* was resistant against all extracts with no or little zone of inhibition.

Introduction

Urinary Tract Infection (UTI) is described as microbial invasion of kidneys, ureters, bladder or urethra and is the second most common clinical symptom for experimental antimicrobial treatment in primary and secondary lane. It is defined as the proliferation of active microorganisms inside the urinary channel which are harmful to their environment (Singh *et al*., 2012). The infecting bacteria normally constitute the faecal flora, the infection is initiated when the urine flow is obstructed by some or other reasons such as calculi, tumours, etc. (Saint *et al*., 2002). The infecting bacteria invade urethra and move to bladder mucosa, multiply and colonize to cause inflammation. Presence of $1x10^5$ or more colonies per millimetre in urine specimens indicates bacteriuria (Stauffer *et al*., 2004). UTI is prevalent in all age groups from neonates to old age and is mostly caused by gram negative bacteria belonging to *Enterobacteriaceae*. If the infection is not controlled, the infecting microbes got resistance to the applied antibiotics intrinsically and a drug resistant cell

survives and predominates with concomitant bacterial genetic exchange mechanisms (McMurry and Levy, 2011). Analysing antibiotic susceptibility pattern of uropathogens helps to overcome the difficulties caused due to antibiotic resistance and guides in choosing appropriate antibiotics (Chowdhury and Parial, 2015). Alternative systems which have great potential for producing new drugs of great benefit to mankind are employed for controlling UTI. This has led to the search of new antibacterial agents with broad spectrum activities from natural sources particularly from medicinal plants. Medicinal plants are valuable sources of novel antibacterials for preventing and controlling microbes. So there is an urgent need to develop antibacterial drugs for UTI.

Materials and Methods

Survey for UTI samples among the urine samples received for culture and sensitivity tests in microbiology lab of Kanyakumari Government Medical College and Hospital, Asaripallam was conducted during August 2014 - July 2015. All the data's regarding the patents, urine culture and patogens were collected and tabulated.

Isolation of UTI bacteria

Microorganisms causing UTI were isolated from the urine samples of infected patients. The urine samples were collected from Microbiology Department of Kanyakumari Medical College and Hospital, Asaripallam. To isolate UTI bacterial strains, samples were serially diluted and a loop full of diluted samples were streaked on MacConkey agar and Nutrient agar plates (Hi Media, India & Merck, Germany) and incubated at 37± 2^{c}c for 24 hrs. After incubation colonies were selected and identified based upon gross colony morphology, standard biochemical tests and also with the help of Bergeys Manual of Systematic Bacteriology (Mac Faddin, 2000, Kreig and Holt, 1984). The identified specimens were streaked on agar slant and stored in refrigerator for further studies.

Plant material: *Aerva lanata* L. Juss., *Scoparia dulcis* L.

Preparation of extract

Plant materials such as *Scoparia dulcis* and *Aerva lanata* were collected from Chunkankadai, Kanyakumari district and washed in running water. Plant parts were separated and again cleaned with distilled water and then dried under shade. Dried materials were then powdered with the help of a mixer grinder. 50g of each powder was packed inside the filter paper and extracted using Methanol and water in a soxhlet apparatus. They were filtered using whatman filter paper No. 42 and concentrated in a rotary evaporator. The concentrated extracts were dried in a water bath and stored in refrigerator for further analysis.

Disc diffusion method

The sensitivity tests were conducted by standard disc diffusion method (Bauer *et al*., 1966). Sub cultures of the tested organisms were made on the previous day. A loop full of isolated colonies was inoculated in to 5ml of nutrient broth in a test tube, incubated for 12 hours at 37°C. This actively growing bacterial cultures were used for antibacterial screening. Pour 15 – 20 ml of Muller Hinton agar medium into sterilized petri plates placed in a laminar air flow hood and allowed to solidify. After 30 minuites the plates were covered, labelled and inverted. Using a sterilized cotton swab the inoculum was evenly spread over the surface of

Muller Hinton agar plate and kept at room temperature for 3-5 minutes for drying. The sterile filter paper discs (6mm) were impregnated with plant extracts (100µml) and left to dry at room temperature. Controls used were ampicillin 10mcg and nitrofuran 300mcg discs. These discs were placed carefully over the surface of the medium using a sterile forceps and gently press to ensure complete contact with the agar surface. Incubate the plates at 37^0C for 24 hours. Following incubation, measure the zone of inhibition to nearest millimetre using a ruler. All the experiments were done in triplicates and the mean zone of inhibition was taken.

Results and Discussion

In the present survey for UTI infections, among the 2177 urine samples obtained during the study period (August 2014- July 2015) for culture and sensitivity tests 621 (28.5%) yield significant bacteriuria. Sex wise analysis showed 60% of the infected samples were from females and 40% from males which showed that the incidence of UTI is higher among females than males. These results are in agreement with the results of similar studies (Chowdhury and Parial, 2015). This may be due to anatomical pre deposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors (Schaffer *et al*., 2001). Females take less amount of water may be another reason. In females UTI commonly occurs in an anatomically normal urinary tract, but in males and children, UTI generally reveals a urinary tract lesion that must be identified by imaging and must be treated to suppress the cause of infection and prevent recurrence (Singh *et al*., 2012). Month wise analysis showed that highest percentage was seen in May (41%) followed by April (37%) and November (31%).The hot climate during these months may cause increased infection. UTI infection was prevalent in all age groups from neonatal to old age. Old age groups (<55) were found to be more susceptible in both males and females (51%, 39%). This is followed by adults in age group 35-55. Least infection was observed in adolescents of age group 13-18. In case of females infections in young adults were more (21%) comparing to males (9%) (Table.1). But in a study by Chowdhury and Parial (2015) highest number of patients with UTI were found in the age group ranging 20 - 40 followed by 1-5 and then the age range 40 - 65.

Among the uropathogens, 29% of infected sample has *E.coli*, 27% contains a mixed population, 20% *Klebsiella*, 10% *Pseudomonas* followed by small percentages of *Proteus*, *Citrobacter*, *Enterococci* etc. So the most common uropathogens in our study were *E.coli* and *Klebsiella*. The previous studies by Ronald (2002), Chowdhury and Parial (2015), Olafsson *et al*., (2000) and Tabassum *et al*., (2013) support this finding. Isolation of UTI pathogens from tribals of West Bengal revealed *E.coli* as the most common bacterium and amikacin as the effective antibiotic (Maji *et al*., 2016). In a survey on UTI associated with the three most common uropathogenic bacteria showed that the first two agents ie, *E.coli* and *Klebsiella* in different seasons were similar while the third one was variable (Behzadi *et al*., 2008). According to Linhares *et al*., (2003) there was difference in bacteria implicated in UTI varied with sex i.e., *Pseudomonas* is common in man than women. Such a difference cannot be seen in the present study.

Multiple antibiotic resistances were reported in UTI and were significantly increased in recent years. Several potent antibiotics are

available for treatment but increasing drug resistance of pathogens has been a great problem for many years. The first bacterium that was detected to be resistant to several antibiotics was reported in Japan during 1950s (Schlegel & Schmidt, 1985). UTI is a complicated problem which has to phase new challenges due to change in etiology and antibacterial resistance of pathogens over years. Extensive use and abuse of antibiotics also contribute to changesin the microbial profile of urinary tract isolates (Mady and Helmi, 2003). So there is an urgent need for new formulation that resists the growth of UTI bacteria. To test the efficiency of selected antiurolithiatic plants, *Aerva lanata* and *Scoparia dulcis* against UTI inducing flora, bacteria were isolated from urine of UTI infected patients. A total of forty urine samples collected from hospital only twenty-five yield bacteriuria. Four types of bacteria were isolated and identified, *E.coli* (60%), *Klebsiella* (16%), *Psuedomonas* (8%) and *Proteus* (4%) and rest were contaminated. Similar UTI pathogens have been reported by Fuad *et al*., (2012), Al-Jiffri *et al*., (2011). Nitrofuran 300 mcg and Ampicillin10mcg were used as standard.

Table.1 Analysis of UTI among the urine samples

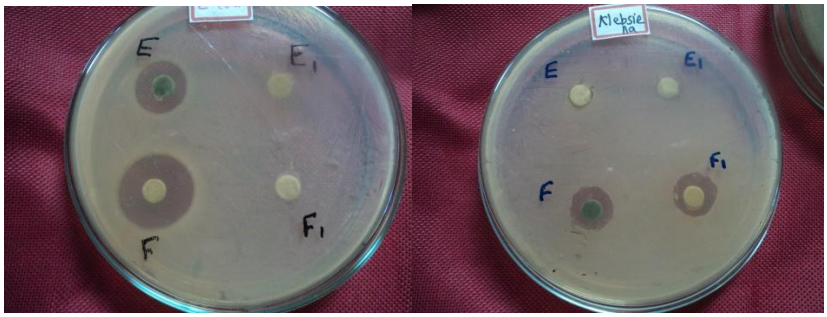
Month	Total no. samples	No. of infected samples	% of infection	Age groups Males						Age groups Females					
				>12	13-18	19-34	35-55	<55	Total	>12	13-18	19-34	35-55	<55	Total
August	163	35	21.47	2	-	1	6	5	14	-	-	6	7	8	21
September	153	49	32.03	5	1	4	2	10	22	-	-	7	5	15	27
October	172	43	25.00	3	-	1	7	10	21	4	-	6	3	9	22
November	200	63	31.50	2	-	2	4	10	18	10	-	11	15	9	45
December	159	38	23.90	1	-	5	5	8	19	1	1	1	7	9	19
January	198	58	29.29	3	-	3	1	12	19	3	-	9	12	15	39
February	148	31	20.95	2	1	-	3	7	13	3	-	5	4	6	18
march	163	42	25.77	3	-	-	6	7	16	2	-	8	6	10	26
April	142	53	37.32	2	-	1	6	9	18	3	1	3	13	15	35
May	163	67	41.10	7	-	1	4	14	26	6	2	7	12	14	41
June	228	72	31.58	7	1	2	8	17	35	4	2	4	9	18	37
July	288	70	24.31	5	-	2	3	18	28	5	1	10	10	16	42
grant total	2177	621	28.53	42	3	22	55	127	249	41	7	77	103	144	372

Table.2 Antimicrobial activities of extracts of *Aerva lanata* and *Scoparia dulcis*

Name of bacteria	Zone of inhibition (mm)													
	Aerva lanata				*Scoparia dulcis*								Control	
	Aerial		Root		Stem		Leaf		Fruit		Root		nitrofuran	Ampicillin
	Aq.	Met.	Aq.	Met.	Aq.	Met	Aq.	Met.	Aq.	Met.	Aq.	met	300mcg	10mcg
E.coli	-	16	-	21.4	-	-	-	-	17.1	20.8	-	-	14.2	15.3
Klebsiella	5.8	6.1	12.00	11.2	-	6.1	-	10.6	-	8.2	7.2	5.8	12.5	7.4
Pseudomonas	-	8.5	8.00	8	-	-	-	14.1	13.2	11.2	-	-	-	17
Proteus	-	-	-	--	-	-	-	6		6.5	-	-	-	-

*All the values are the mean of triplicate experiments

Fig.1 E.*coli* Figure:2 *Klebsiella*



E-*Aerva* aerial Methanol extract E_1-*Aerva* aerial Aqueous extract
F-A root Methanol extract F_1-*Aerva* root Aqueous extract

The results of antibacterial analysis showed that the extracts of *Aerva lanata* were more susceptible to clinical isolates of UTI pathogens. The zone of inhibition of methanol extract of *Aerva lanata* root (21.3mm) and *Scoparia* fruit (20.6) showed higher activity than that of control nitrofuran and ampicillin (14.2 and 15.3) while *Aerva* aerial parts showed 15.5mm inhibition against *E.coli*. There for the whole plant of *Aerva lanata* and fruit of *Scoparia dulcis* has the power to inhibit *E.coli*. In case of *Scoparia* the fruit extract was found to be effective against all tested bacteria having the highest zone of inhibition 20.8 against *E.coli*. *Klebsiella* has zone of inhibition in all tested plant parts. *Pseudomonas* showed zone of inhibitions against *Aerva* parts, *Scoparia* leaf and fruit. *Proteus*, a less common UTI bacterium was found to be resistant to all the extract with little or no zone of inhibition (Table 2, Figures1-2)

Muller Hinton agar appears to be the best medium for antibacterial susceptibility tests and the same was used in the present study. Methanolic extracts showed more activity than aqueous extracts. This may be due to higher activation or solubility of biologically active compounds such as alkaloids, flavonoids, terpenoids etc. in organic solvents (de Boer *et al*., 2005). Antibacterial activity of *Euphorbia hirta*, *Erythrophelum suaveolens* and *Thevetia peruviana* against UTI causing bacteria showed highest activity in methanolic extracts (Singh *et al*., 2012). In another study ethanolic extract of *Scoparia dulcis* showed activity against *E.coli*, *Styphylococcus aureus* and *Shigella dysenteriae* (Uddin *et al*., 2014).

Susceptibility tests for UTI bacteria showed highest degree of resistance to gentamycin, nalidixic acid, trimethoprim sulpha-methaoxazole, clotrimazole and cefotaxime

which are commonly prescribed for UTI treatment. The antibiotics which are effective up to some extent were ampicillin, norfloxacin and tetracycline (Tabassum *et al*., 2013).

According to Valsalakumari *et al*., (2014) ethanolic extracts of *Scoparia dulcis* showed zone of inhibition only for gram positive bacteria, while gram negative *E.coli* was resistant. In another study using the same extract by Mohandas *et al*., (2014) against human pathogens *Streptococcus* (gram positive) and *Pseudomonas* (gram negative) showed zones of inhibition for both bacteria suggested the presence of broad spectrum antibiotic compound. In the present study the fruit methanolic extract had significant zone of inhibition.

The samples tested for antimicrobial activity in this study may have some bioactive compounds that were potent and further investigations were needed to identify the compound in pure form.

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