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Antimicrobial effect of drugs, medicinal plant extracts and essential oils against Pathogenic Bacteria causing Urinary Tract Infection

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ABSTRACT

Urinary tract infection (UTI) is a term applied to a variety of clinical conditions ranging from asymptomatic presence of bacteria in the urine to severe infection of the kidney with resultant sepsis. UTI is defined also as the growth of a known bacterial pathogen more than 10000 cfu/ml in association with a positive dipstick or urinalysis. Urinary tract infections (UTIs) are a serious health problem affecting millions of people each year. Infections of the urinary tract are the second most common type of infection in the body. These are one of the most common bacterial infections affecting humans throughout their life span. Most of urinary tract infections are caused by gram-negative bacteria like *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter* and *Serratia*. 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram-positive bacteria. Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents; hence resistance to old and newly produced drugs is on the rise. The phenomenon of antibiotic resistance exhibited by the pathogenic microorganisms has led to the need for screening of several medicinal plants for their potential antimicrobial activity. The present study was conducted to identify the uropathogens based on morphological and biochemical characteristics and to study the antimicrobial effect of drugs, medicinal plant extracts and essential oils against uropathogens.

Introduction

UTI encompass a spectrum of clinical entities ranging in severity from asymptomatic infection to acute cystitis, prostatitis, pyelonephritis and urithritis (Abubakar, 2009). It represents one of the most common diseases encountered in medical practice today, affecting people of all ages, from the neonate to the geriatric age group (Al-Jiffri, *et al*., 2011). Most infections are caused by retrograde ascent of bacteria from the faecal flora *via* the urethra to the bladder and kidney especially in the females who have a shorter and wider urethra and are more readily transversed by microorganisms (Borchert, et al., 2008). The Clinical symptoms of UTI usually include frequency, dysuria, pyuria, abdominal pain, back pain, fever or urgency. But none of these symptoms alone is sufficient to establish UTI diagnosis in human (Bonadio, *et al*.,1999). The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria been massaged up the urethra and into the bladder during pregnancy and or child birth (El-Sweih, *et al*., 2008). Most of urinary tract infections are caused by gram-negative bacteria like *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Acinetobacter* and *Serratia*. 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by grampositive bacteria. The most common etiological agent of uncomplicated UTI is *E.coli*, which is present in about 80%-90% of cases (Choi, et al., 2009).Gram-positive bacteria include *Enterococcus*, *Staphylococcus* and *Streptococcus agalactiae* (Geerlings, *et al*.,2002). Most UTIs in children are monomicrobic, often caused by *Escherichia coli* (60 to 80 percent of cases), *Proteus*, *Klebsiella*, *Enterococcus* and coagulase negative *Staphylococci* (Hamood, *et al*., 1996)). *Escherichia coli* are the most common gram-negative bacteria responsible for UTI (Justice, *et al*., 2004).

The spiraling costs of antibiotic therapy, the appearance of multi resistant bacteria and more importantly for patients and clinicians, unsatisfactory therapeutic options in recurrent urinary tract infection (RUTI) calls for alternative and advanced medical solutions. So far no sufficient means to successfully prevent painful and disabling RUTI has been found. Even though long-term oral antibiotic treatment has been used with some success as a therapeutic option, this is no longer secure due to the development of bacterial resistance. One promising alternative is the use of live microorganisms (probiotics) to prevent and treat recurrent complicated and uncomplicated urinary tract infection (Malik, and Singh, 2010).

Material and Methods

The present study was carried out in Department of Biomedical Sciences, Dolphin (PG) Institute of Biomedical & Natural Sciences, Dehradun.

Collection of Samples

A total of 50 urine samples were collected aseptically from different patients of Doon and C.M.I. Hospital, Dehradun and transported to lab.

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Recovery of Isolates

Isolates were recovered by plating on C.L.E.D agar, Blood Agar, EMB agar, Dettol agar and Mc Conkey agar. T-streaking method was used for plating on C.L.E.D agar and Blood agar using calibrated loop. Plates were incubated at 37 ± 1^0C. The samples in which bacterial count is > 10^5 cfu/ml were taken for further processing. All samples were plated in triplicates (Dickinson and Bisno, 1989).

Purification, Maintenance and Preservation of Cultures

Isolates were purified by streaking on Nutrient agar and pure cultures were maintained. Glycerol stocks were prepared by adding 5.0 ml of autoclaved glycerol to 5 ml of overnight grown culture in Nutrient broth. Glycerol stocks were maintained in cryovials and preserved at -20^0C (Goldsworthy, 2008).

Morphological Characterization

Cell Morphology (Gram's reaction, cell shape and arrangement) of isolates were studied.

Biochemical Characterization

The various biochemical tests viz., Oxidase test, Indole-Methyl Red –Voges-Proskauer-Citrate Utilization test (IMViC), Triple Sugar Iron (TSI) Test and Nitrate reduction tests were carried out according to Cappucino and Sherman(1992) by using different media (Litwin, *et al*., 2005).

Antibiotic Sensitivity Assay

All isolated were tested for antibiotic sensitivity by Kirby-Bauer disc diffusion method () on Mueller-Hinton agar. Cultures were inoculated by swabbing with standard inoculum over the entire agar surface. The agar surface was allowed to dry for 3-5 minutes before applying the antibiotic discs using sterile forcep. Four Antibiotic discs were placed equidistantly on 90 mm Petriplate. The Plates were incubated aerobically at 37 ± 1^0C in incubator for 16 to 18 hrs. Next day the zone of inhibition was measured.

Antibiotics used were: AC- Amoxycillin (30mcg); AK- Amikacin (30mcg); CO- Cotrimaxazole (1.25/23.75mcg); CS- Cefoperazone/Sulbactam (50/50mcg); CE- Cefotaxime (30 mcg); CF- Cefoperazone (75mcg).

Antimicrobial Activity of Medicinal Plants

The antimicrobial activity of aqueous and ethanolic extracts of 3 medicinal plants viz., peel of *Punica granatum*, *Stevia* and *Allium sativum* were tested against recovered isolates. The extracts were diluted 1:2, 1:5 and 1:10 in autoclaved distilled water and ethanol. Cultures were inoculated by swabbing with standard inoculum over the entire agar surface. The agar surface was allowed to dry for 3-5 minutes before applying the sterile discs using sterile forcep. 20 µl of Neat, 1:2, 1:5 and 1:10 dilutions were put on sterile discs and allowed the discs to absorb. The discs were placed equidistantly on 90 mm Petri plate along with control. The Plates were incubated aerobically at 37 ± 1^0C in incubator for 16 to 18 hrs. Next day the zone of inhibition was measured (Kolawale, *et al*., 2009).

Antimicrobial Activity of Essential Oils

The antimicrobial activity of 4 essential oils viz., Lemon grass oil, Basil oil, Japanese mint oil and Germanium oil were tested against recovered isolates. The oils were diluted 1:2, 1:5 and 1:10 in autoclaved DMSO. Cultures were inoculated by swabbing with standard inoculum over the entire agar surface. The agar surface was allowed to dry for 3-5 minutes before applying the sterile discs using sterile forcep. 20 µl of Neat, 1:2, 1:5 and 1:10 dilutions were put on sterile discs and allowed the discs to absorb. The discs were placed equidistantly on 90 mm Petriplate along with control. The Plates were incubated aerobically at 37 ± 1^0C in incubator for 16 to 18 hrs. Next day the zone of inhibition was measured (Kalantar, *et al*., 2008).

Results

The present study was carried out to study the prevalence of uropathogens and to characterise them.

Prevalence of Uropathogens

A total of 90 uropathogens were obtained from positive urine samples (Fig. 1) which were identified based on morphological (Table 1) and biochemical characteristics (Table 2). *E. coli* was the most prevalent uropathogen followed by *Pseudomonas* (22%), *Proteus* (14%), *Serratia* (16%), *Staphylococcus* (6%) and *Klebsiella* (4%).

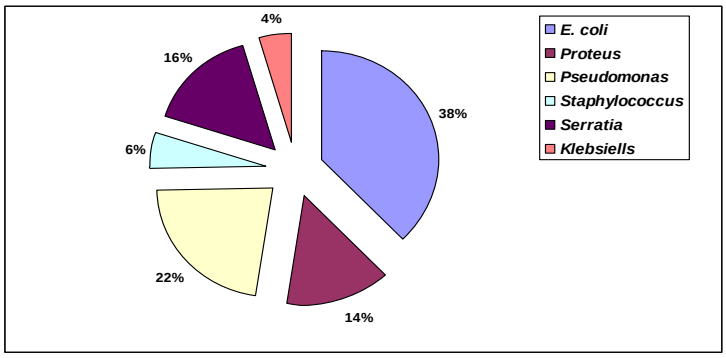

Fig. 1: Distribution of Uropathogens

Antibiotic Sensitivity Assay

Fig. 2 showed Isolates of *E. coli* were found to be maximally sensitive towards Cefoperazone (25%) followed by Cefotaxime (22%) and Cotrimaxazole (22%).

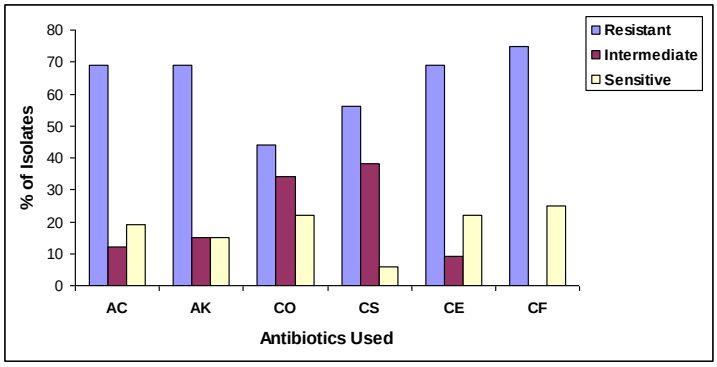

Fig. 2: Antimicrobial susceptibility of antibiotics against *E.coli*

AC- Amoxycillin; AK- Amikacin; CO- Cotrimaxazole; CS- Cefoperazone/Sulbactam; CE- Cefotaxime; CF- Cefoperazone

Fig.3 showed Isolates of *Pseudomonas* were found to maximally sensitive towards Cefoperazone/Sulbactam (25%) followed by Cefotaxime (20%), Amikacin (15%).

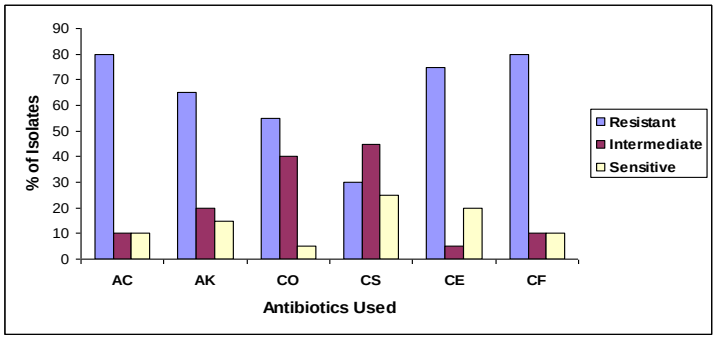

Fig. 3: Antimicrobial susceptibility of antibiotics against *Pseudomonas*

AC- Amoxycillin; AK- Amikacin; CO- Cotrimaxazole; CS- Cefoperazone/Sulbactam; CE- Cefotaxime; CF- Cefoperazone.

Fig. 4 showed Isolates of *Serratia* were found to maximally sensitive towards Amikacin (36%) followed by Cotrimaxazole (21%) and Amoxycillin (14%).

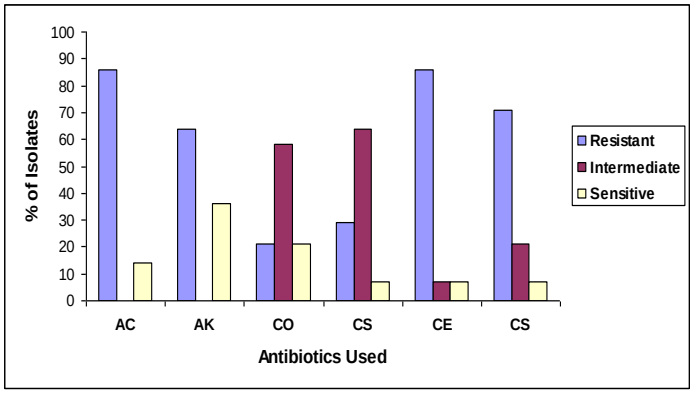

Fig. 4: Antimicrobial susceptibility of antibiotics against *Serratia*

AC- Amoxycillin; AK- Amikacin; CO- Cotrimaxazole; CS- Cefoperazone/Sulbactam; CE- Cefotaxime; CF- Cefoperazone

Fig.5 showed Isolates of *Staphylococcus* were found to maximally sensitive towards Cefotaxime (29%) followed by Amikacin (14%), Cotrimaxazole (14%), Cefoperazone (14%) and Amoxycillin (14%).

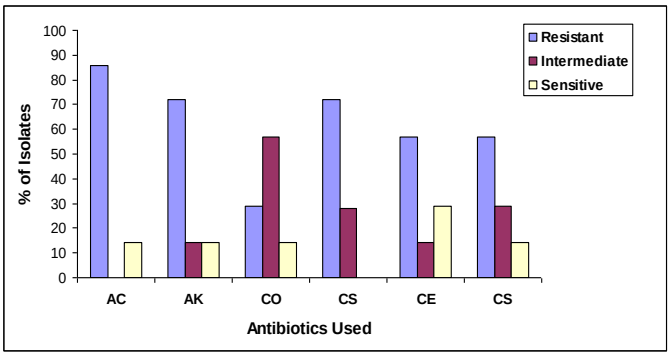

Fig. 5: Antimicrobial susceptibility of antibiotics against *Staphylococcus*

AC- Amoxycillin; AK- Amikacin; CO- Cotrimaxazole; CS- Cefoperazone/Sulbactam; CE- Cefotaxime; CF- Cefoperazone

Fig.6 showed Isolates of *Proteus* were found to maximally sensitive towards Cotrimaxazole (31%), followed by Cefotaxime (16%).

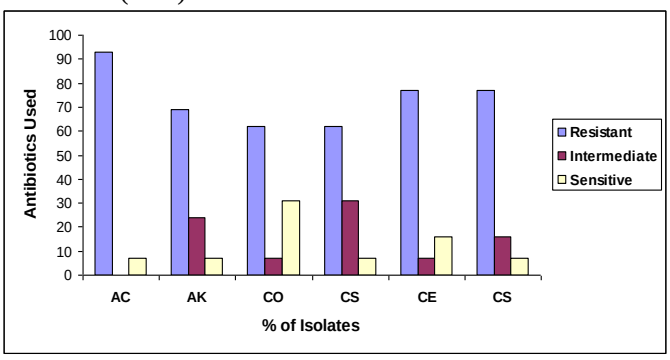

Fig. 6: Antimicrobial susceptibility of antibiotics against *Proteus*

AC- Amoxycillin; AK- Amikacin; CO- Cotrimaxazole; CS- Cefoperazone/Sulbactam; CE- Cefotaxime; CF- Cefoperazone

Fig.7 showed Isolates of *Klebsiella* were found to maximally sensitive towards Amoxycillin (25%) and Amikacin(25%).

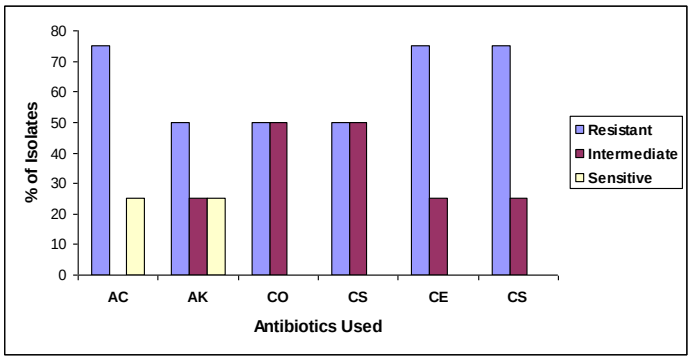

Fig. 7: Antimicrobial susceptibility of antibiotics against *Klebsiella*

AC- Amoxycillin; AK- Amikacin; CO- Cotrimaxazole; CS- Cefoperazone/Sulbactam; CE- Cefotaxime; CF- Cefoperazone

Antimicrobial activity of medicinal plants against uropathogens

Proteus isolates were found to be most sensitive towards aqueous extract of *Allium sativum* and ethanolic extracts of *Stevia* (Table 3). *Pseudomonas* isolates were found to be most sensitive towards aqueous extract of *Allium sativum* and ethanolic extracts of *Stevia*. *Klebsiella* isolates were found to be most sensitive towards aqueous extract of *Stevia* and ethanolic extracts of *Allium sativum*. *E. coli* isolates were found to be most sensitive towards aqueous extract of *Punica granatum* and ethanolic extracts of *Stevia*. *Serratia* isolates were found to be most sensitive towards aqueous extract of *Allium sativum* and ethanolic extracts of *Stevia*. *Staphylococcus* isolates were found to be most sensitive towards aqueous extract of *Stevia* and ethanolic extracts of *Punica granatum*.

Antimicrobial activity of essential oils against uropathogens

Isolates of *Proteus* were found to be sensitive towards only basil oil while isolates of *Pseudomonas* and *E. coli* were found to be sensitive towards all oils used (Table 4). *Pseudomonas* and *E. coli* were found to be most sensitive towards germanium oil. Isolates of *Klebsiella* were found to be most sensitive towards lemon grass oil and germanium oil. *Serratia* was observed to be most sensitive towards Japanese mint oil. *Staphylococcus* was found to be most sensitive towards germanium oil.

Fig.8

Fig.9

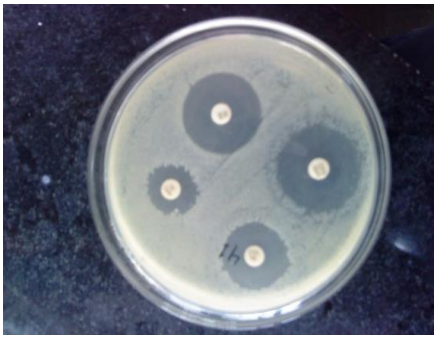

Fig.10

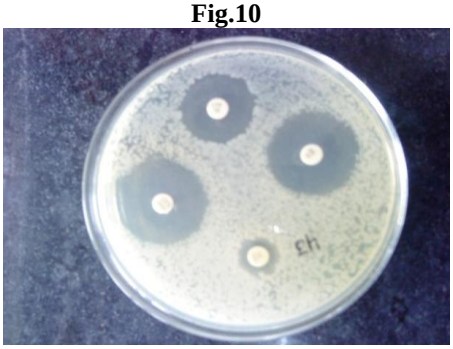

Fig.11

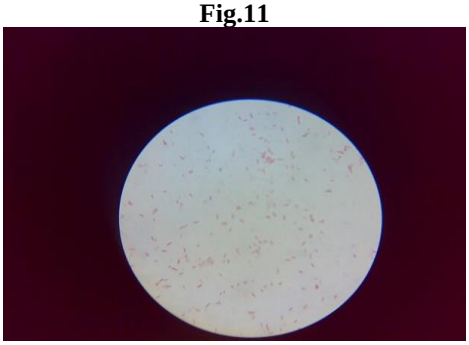

Fig.12

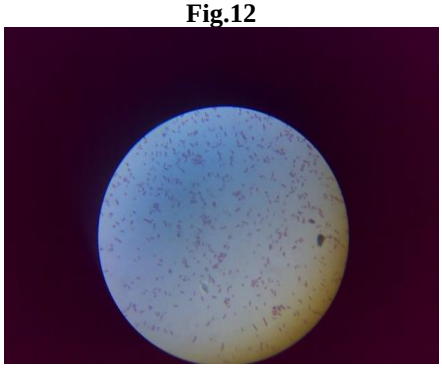

Fig.13

Figures 8,9,10 and 11 showed the inhibition zones and Figures 12 and 13 showed bacterial strains

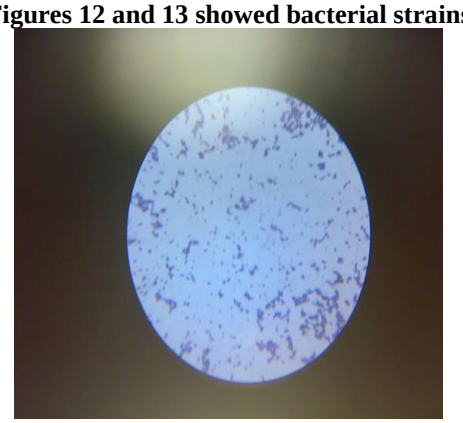

Fig.14

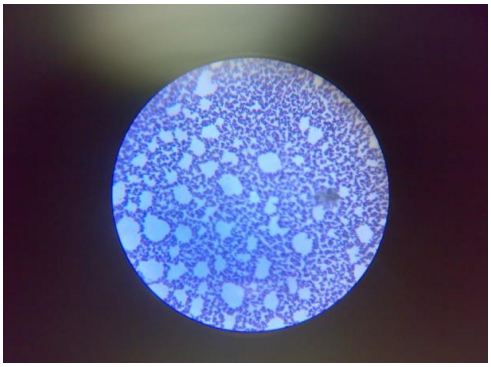

Fig.15

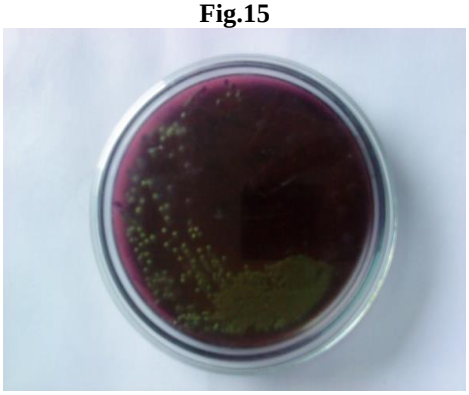

Fig.16

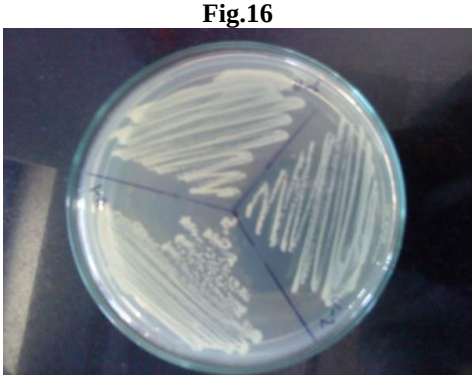

Fig.17

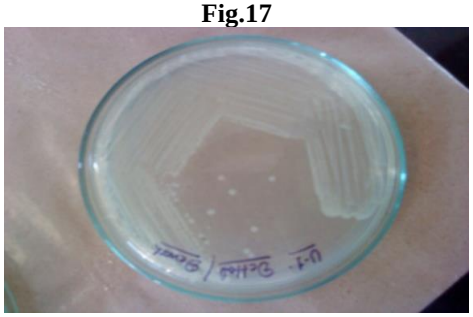

Fig.18

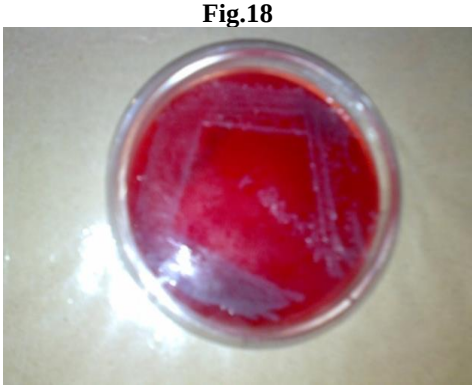

Fig.19

Figures 14 and 15 showed bacterial strains and figures 16,17, 18 and 19 showed bacterial colonies

Table 1: Morphological Characteristics of Recovered Uropathogens

Isolates	Gram's Reaction	Cell Shape	Cell Arrangement
U 1	-	Short rod	Isolated
U 2	-	Rod	Isolated
U 3	-	-	-
U 4	+	+	-
U 5	+	+	-
U 6	+	+	-
U 7	-	Rod	Isolated
U 8	+	+	-
U 9	-	-	-
U 10	-	Rod	Isolated
U 11	-	-	-
U12	-	-	-
U13	-	-	+
U 14	-	-	+
U 15	-	-	+
U 16	+	+	-
U 17	-	-	+
U18	-	+	-
U19	+	+	-
U20	+	+	-
U21	+	+	-
U22	-	-	+
U23	-	+	+
U24	+	+	-
U25	-	+	+
U26	+	+	-
U27	-	-	+
U28	-	-	+
U29	+	+	-
U30	-	-	-
U31	+	+	-
U32	-	Rod	Isolated
U33	+	+	-
U34	+	+	-
U35	-	-	+
U36	+	+	-
U37	-	-	+
U38	+	+	-
U39	+	+	-
U40	-	-	+
U41	+	cocci	Isolated
U42	+	+	-
U43	-	-	+
U44	+	+	-
U45	+	+	-
U46	-	-	-
U47	-	-	+
U48	-	-	+
U49	+	+	-
U50	+	+	-
U51	-	-	-
U52	-	-	+
U53	+	+	-
U54	+	+	-
U55	-	-	+
U56	+	+	-
U57	-	-	-
U58	+	+	-
U59	+	+	-
U60	-	-	-
U61	+	+	-
U62	-	-	-
U63	-	+	+
U64	+	+	-
U65	-	+	+

U66	-	-	-
U67	-	-	+
U68	-	-	-
U69	+	+	-
U70	+	+	-
U71	-	-	-
U72	-	+	+
U73	+	+	-
U74	-	-	-
U75	+	+	-
U76	+	+	-
U77	-	-	-
U78	+	+	-
U79	+	+	-
U80	+	+	-
U81	-	-	-
U82	+	+	-
U83	-	-	-
U84	-	-	-
U85	+	+	-
U86	-	-	-
U87	+	+	-
U88	+	+	-
U89	+	+	-
U90	+	+	-

Table 2: Identification of Recovered Isolates Based on Biochemical Tests

Isolates	I	MR	VP	C	TSI	NITRATE	OXIDASE	Identified Organism
U 1	+	+	-	-	A/A	+	-	*Escherichia coli*
U 2	-	-	-	+	K/K	+	+	*Pseudomonas*
U 3	-	-	-	+	K/K	+	+	*Pseudomonas*
U 4	+	+	-	-	K/A	+	-	*Proteus*
U 5	+	+	-	-	A/A	+	-	*Escherichia coli*
U 6	+	+	-	-	K/A	+	-	*Proteus*
U 7	-	-	+	+	A/A	+	-	*Klebsiella*
U 8	+	+	-	-	A/A	+	-	*Escherichia coli*
U 9	-	-	-	+	K/K	+	+	*Pseudomonas*
U 10	+	+	-	-	K/A	+	-	*Proteus*
U 11	-	-	-	+	K/K	+	+	*Pseudomonas*
U12	-	-	-	+	K/K	+	+	*Pseudomonas*
U13	-	-	+	+	A/A	+	-	*Klebsiella*
U 14	-	-	+	+	A/A	+	-	*Klebsiella*
U 15	-	-	+	+	A/A	+	-	*Klebsiella*
U 16	+	+	-	-	K/A	+	-	*Proteus*
U 17	-	-	+	+	K/A	+	-	*Serratia*
U18	-	+	-	-	A/A	+	-	*Staphylococcus*
U19	+	+	-	-	K/A	+	-	*Proteus*
U20	+	+	-	-	A/A	+	-	*Escherichia coli*
U21	+	+	-	-	A/A	+	-	*Escherichia coli*
U22	-	-	+	+	K/A	+	-	*Serratia*
U23	-	+	+	+	A/A	+	-	*Staphylocoocus*
U24	+	+	-	-	A/A	+	-	*Escherichia coli*
U25	-	+	+	-	A/A	+	-	*Staphylococcus*
U26	+	+	-	-	A/A	+	-	*Escherichia coli*
U27	-	-	+	+	K/A	+	-	*Serratia*
U28	-	-	+	+	K/A	+	-	*Serratia*
U29	+	+	-	-	K/A	+	-	*Proteus*
U30	-	-	-	+	K/K	+	+	*Pseudomonas*
U31	+	+	-	-	A/A	+	-	*Escherichia coli*
U32	-	-	+	+	K/A	+	-	*Serratia*
U33	+	+	-	-	K/A	+	-	*Proteus*
U34	+	+	-	-	A/A	+	-	*Escherichia coli*
U35	-	-	+	+	K/A	+	-	*Serratia*
U36	+	+	-	-	K/A	+	-	*Proteus*
U37	-	-	+	+	K/A	+	-	*Serratia*
U38	+	+	-	-	A/A	+	-	*Escherichia coli*
U39	+	+	-	-	A/A	+	-	*Escherichia coli*

U40	-	-	+	+	K/A	+	-	*Serratia*
U41	-	+	+	-	A/A	+	-	*Staphylococcus*
U42	+	+	-	-	A/A	+	-	*Escherichia coli*
U43	-	-	+	+	K/A	+	-	*Serratia*
U44	+	+	-	-	A/A	+	-	*Escherichia coli*
U45	+	+	-	-	A/A	+	-	*Escherichia coli*
U46	-	-	-	+	K/K	+	+	*Pseudomonas*
U47	-	-	+	+	K/A	+	-	*Serratia*
U48	-	-	+	+	K/A	+	-	*Serratia*
U49	+	+	-	-	A/A	+	-	*Escherichia coli*
U50	+	+	-	-	A/A	+	-	*Escherichia coli*
U51	-	-	-	+	K/K	+	+	*Pseudomonas*
U52	-	-	+	+	K/A	+	-	*Serratia*
U53	+	+	-	-	A/A	+	-	*Escherichia coli*
U54	+	+	-	-	A/A	+	-	*Escherichia coli*
U55	-	-	+	+	K/A	+	-	*Serratia*
U56	+	+	-	-	K/A	+	-	*Proteus*
U57	-	-	-	+	K/K	+	+	*Pseudomonas*
U58	+	+	-	-	A/A	+	-	*Escherichia coli*
U59	+	+	-	-	K/A	+	-	*Proteus*
U60	-	-	-	+	K/K	+	+	*Pseudomonas*
U61	+	+	-	-	K/A	+	-	*Proteus*
U62	-	-	-	+	K/K	+	+	*Pseudomonas*
U63	-	+	+	-	A/A	+	-	*Staphylococcus*
U64	+	+	-	-	A/A	+	-	*Escherichia coli*
U65	-	+	+	-	A/A	+	-	*Staphylococcus*
U66	-	-	-	+	K/K	+	+	*Pseudomonas*
U67	-	-	+	+	K/A	+	-	*Serratia*
U68	-	-	-	+	K/K	+	+	*Pseudomonas*
U69	+	+	-	-	A/A	+	-	*Escherichia coli*
U70	+	+	-	-	K/A	+	-	*Proteus*
U71	-	-	-	+	K/K	+	+	*Pseudomonas*
U72	-	+	+	-	A/A	+	-	*Staphylococcus*
U73	+	+	-	-	A/A	+	-	*Escherichia coli*
U74	-	-	-	+	K/K	+	+	*Pseudomonas*
U75	+	+	-	-	A/A	+	-	*Escherichia coli*
U76	+	+	-	-	A/A	+	-	*Escherichia coli*
U77	-	-	-	+	K/K	+	+	*Pseudomonas*
U78	+	+	-	-	A/A	+	-	*Escherichia coli*
U79	+	+	-	-	A/A	+	-	*Escherichia coli*
U80	+	+	-	-	A/A	+	-	*Escherichia coli*
U81	-	-	-	+	K/K	+	+	*Pseudomonas*
U82	+	+	-	-	A/A	+	-	*Escherichia coli*
U83	-	-	-	+	K/K	+	+	*Pseudomonas*
U84	-	-	-	+	K/K	+	+	*Pseudomonas*
U85	+	+	-	-	A/A	+	-	*Escherichia coli*
U86	-	-	-	+	K/K	+	+	*Pseudomonas*
U87	+	+	-	-	A/A	+	-	*Escherichia coli*
U88	+	+	-	-	A/A	+	-	*Escherichia coli*
U89	+	+	-	-	K/A	+	-	*Proteus*
U90	+	+	-	-	A/A	+	-	*Escherichia coli*

Table 3: Antimicrobial activity of medicinal plants against uropathogens

Name of Isolate	Name of Medicinal Plant	Fraction Used	Dilutions Used			
			1:2	1:5	1:10	NEAT
Proteus	*Stevia*	Aqueous	2±0.3	3±0.1	5±0.2	1±0.1
	Allium sativum		8±0.2	15±1	12±1	7±1
	Punica granatum		9±1	6±2	5±0.2	5±0.1
	Stevia	Ethanolic	36±2	25±1	15±1	No zone
	Allium sativum		12±0.2	36±1	6±1	No zone
	Punica granatum		36±0.3	16±1	11±1	32±2
Pseudomonas	*Stevia*	Aqueous	1±0.1	2±1	1±0.1	1±0.1
	Allium sativum		24±2	15±1	12±1	26±2
	Punica granatum		5±0.2	No zone	No zone	1±0.1
	Stevia	Ethanolic	14±1	8±0.2	5±0.2	23±1
	Allium sativum		7±1	14±2	18±2	1±0.2
	Punica granatum		8±0.4	No zone	No zone	11±1

Klebsiella	*Stevia*	Aqueous	13±1	6±1	5±1	5±0.2
	Allium sativum		5±0.1	4±0.3	3±1	4±0.3
	Punica granatum		5±0.4	6±1	3±0.1	5±0.3
	Stevia	Ethanolic	12±1	8±0.2	4±0.1	21±1
	Allium sativum		14±1	26±2	34±1	3±1
	Punica granatum		12±0.4	6±0.3	4±0.1	15±1
E. coli	*Stevia*	Aqueous	16±1	5±0.2	1±0.1	4±0.2
	Allium sativum		12±1	8±0.2	4±0.1	3±0.1
	Punica granatum		18±2	26±1	10±1	12±1
	Stevia	Ethanolic	8±0.3	6±0.1	1±0.1	13±1
	Allium sativum		No zone	No zone	No zone	12±2
	Punica granatum		8±1	6±1	1±0.2	12±1
Serratia	*Stevia*	Aqueous	8±1	7±1	6±1	9 ±0.3
	Allium sativum		23±2	16±1	8±0.1	12±1
	Punica granatum		11±1	23±2	No zone	13±1
	Stevia	Ethanolic	18±1	26±2	12±1	13±1
	Allium sativum		6±1	5±1	3±1	12±1
	Punica granatum		12±2	No zone	No zone	8±1
Staphylococcus	*Stevia*	Aqueous	No zone	No zone	No zone	5±0.2
	Allium sativum		No zone	No zone	No zone	No zone
	Punica granatum		No zone	No zone	No zone	No zone
	Stevia	Ethanolic	No zone	No zone	No zone	No zone
	Allium sativum		No zone	No zone	No zone	No zone
	Punica granatum		5±0.5	5±0.2	1±0.1	7±0.3

Table 4: Antimicrobial activity of essential oils against uropathogens

Name of Isolate	**Name of Oil used**	**Dilutions Used**			
		1:2	1:5	1:10	NEAT
Proteus	Lemon grass oil	No zone	No zone	No zone	No zone
	Basil oil	36±1	12±0.5	2±0.4	43±2
	Japanese mint oil	No zone	No zone	No zone	No zone
	Germanium oil	No zone	No zone	No zone	No zone
Pseudomonas	Lemon grass oil	No zone	No zone	No zone	4±0.7
	Basil oil	5±1	13±2	9±1	5±0.5
	Japanese mint oil	5±0.6	5±0.3	1±0.1	7±1
	Germanium oil	5±0.4	4±0.2	3±1	9±0.4
E. coli					
	Lemon grass oil	5±0.3	5±0.2	1±0.1	5±0.4
	Basil oil	4±0.1	2±0.1	1±0.1	5±0.4
	Japanese mint oil	3±1	2±1	1±0.5	3±0.1
	Germanium oil	5±0.3	4±0.2	1±0.1	11±0.7
Klebsiella	Lemon grass oil	No zone	No zone	No zone	21±2
	Basil oil	No zone	No zone	No zone	No zone
	Japanese mint oil	No zone	No zone	No zone	No zone
	Germanium oil	No zone	No zone	No zone	21±3
Serratia	Lemon grass oil	2±0.4	No zone	No zone	6±1
	Basil oil	No zone	No zone	No zone	5±1
	Japanese mint oil	16±1	7±1	3±0.2	36±2
	Germanium oil	1±0.3	No zone	No zone	1±0.1
Staphylococcus	Lemon grass oil	4±1	No zone	No zone	4±1
	Basil oil	5±1	4±0.1	2±0.3	6±2
	Japanese mint oil	5±2	4±0.3	3±0.1	6±0.5
	Germanium oil	16±1	8±2	No zone	12±2

Discussion

In the present study *E. coli* was the most prevalent uropathogen followed by *Pseudomonas*, *Proteus*, *Serratia*, *Staphylococcus* and *Klebsiella*. The most common etiological agent of uncomplicated UTI is *E.coli*, which is present in about 80%-90% of cases (Leone, *et al*., 2003)). 90% of UTI cases are caused by gram-negative bacteria while only 10% of the cases are caused by gram positive bacteria. Gram-positive bacteria include *Enterococcus, Staphylococcus* and *Streptococcus agalactiae* (Lawrence, *et al*., 2009). Most UTIs in children are monomicrobic, often caused by *Escherichia coli* (60 to 80 percent of cases), *Proteus*, *Klebsiella*, *Enterococcus* and coagulase negative *Staphylococci* (Sharma, *et al*., 2009). *Escherichia coli* are the most common gram-negative bacteria responsible for UTI (Onyeagba, *et al*., 2004). At least 80% of the uncomplicated cystitis and pyelonephritis are due to *Escherichia coli* (Prusti, *et al*., 2008)) whereas *Proteus mirabilis* and *Klebsiella pneumoniae* infections accounts 10% and 6% respectively (Woods, *et al*., 1986).

The study of virulence factors of uropathogens would enable to devise treatment strategy. Isolates of *E. coli* were found to be maximally sensitive towards Cefoperazone (25%) followed by Cefotaxime (22%) and Cotrimaxazole (22%). Isolates of *Pseudomonas* were found to maximally sensitive towards Cefoperazone/Sulbactam (25%) followed by Cefotaxime (20%), Amikacin (15%). Isolates of *Serratia* were found to maximally sensitive towards Amikacin (36%) followed by Cotrimaxazole (21%) and Amoxycillin (14%). Isolates of *Staphylococcus* were found to maximally sensitive towards Cefotaxime (29%) followed by Amikacin (14%), Cotrimaxazole (14%), Cefoperazone (14%) and Amoxycillin (14%). Isolates of *Proteus* were found to maximally sensitive towards Cotrimaxazole (31%), followed by Cefotaxime (16%). Isolates of *Klebsiella* were found to maximally sensitive towards Amoxycillin (25%) and Amikacin (25%). Even though long-term oral antibiotic treatment has been used with some success as a therapeutic option, this is no longer secure due to the development of bacterial resistance.(Saritha, *et al*., 2010) reported that 37% *E. coli* strains were resistant to amoxycillin+clavulanate 33% to cotrimoxazole and 22% to ciprofloxacin. Seven strains of *E. coli* produced ESBL. Thirteen per cent of strains were resistant to cefuroxime but only (1%) to fosfomycin. Resistance to nitrofurantoin in *K. pneumoniae* was 38%. *P. mirabilis* showed 52% resistance to cotrimoxazole and 13% *Staphylococcus aureus*, were methicillin-resistant. *E. faecalis* did not show any special resistance to normal medication. Fosfomycin continued to show high activity against Gram-negative bacilli (Sahoo, *et al*., 2008). However, enterococci, some species of *Staphylococci* and yeasts were difficult to treat empirically. ESBL were detected in the isolates of *E. coli* and there were some methicillin-resistant strains of *S. aureus* (Yates, *et al*., 2006).

Proteus isolates were found to be most sensitive towards aqueous extract of *Allium sativum* and ethanolic extracts of *Stevia*. *Pseudomonas* isolates were found to be most sensitive towards aqueous extract of *Allium sativum* and ethanolic extracts of *Stevia*. *Klebsiell* isolates were found to be most sensitive towards aqueous extract of *Stevia* and ethanolic extracts of *Allium sativum*. *E. coli* isolates were found to be most sensitive towards aqueous extract of *Punica granatum* and ethanolic extracts of *Stevia*. *Serratia* isolates were found to be most sensitive towards aqueous extract of *Allium sativum* and ethanolic extracts of *Stevia*. *Staphylococcus* isolates were found to be most sensitive towards aqueous extract of *Stevia* and ethanolic extracts of *Punica granatum.* Zulianello, *et al*. (2006) tested arils from six pomegranate (*Punica granatum* L.) varieties grown in the Mediterranean region of Turkey for their antimicrobial properties by the agar diffusion and minimum inhibitory concentration (MIC) methods against seven bacteria: (*Bacillus megaterium* DSM 32, *Pseudomonas aeruginosa* DSM 9027, *Staphylococcus aureus* Cowan 1, *Corynebacterium xerosis* UC 9165, *Escherichia coli* DM, *Enterococcus faecalis* A10, *Micrococcus luteus* LA 2971), and three fungi (*Kluveromyces marxianus* A230, *Rhodotorula rubra* MC12, *Candida albicans* ATCC 1023). The pomegranate aril extracts had antimicrobial effect on all microorganisms, giving inhibition zones ranging in size from 13 to 26 mm. The MIC values for active pomegranate extracts ranged between 30 and >90 µg/mL (Nicolle, 2008).

Punica granatum is commonly used in Korea as a traditional medicine for the treatment of pathogenic bacteria.) Matheson *et al*. (2006) investigated the *in vitro* and *in vivo* antimicrobial activity of *P. granatum* peel EtOH extract (PGPE) against 16 strains of *Salmonella*.) tested the antibacterial activity of garlic powder against O-157 by using garlic bulbs post-harvested 1 year. O-157 at 10 (6-7) CFU/ ml perished after incubation for 24 h with a 1% solution of garlic powder. The use of powder from fresh garlic was more effective for antibacterial activity than that from old garlic; the 1% solution of fresh garlic powder eradicating the O-157 in 6 h (Hootan, 2001). The antibacterial activity was also shown against other types of pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella enteritidis*, and *Candida albicans*.) studied the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn) against *Staphylococcus aureus*, *Bacillus* spp., *Escherichia coli* and *Salmonella* spp (Kalsi, et al., 2003). All the test organisms were susceptible to undiluted lime-juice (Mittal, *et al*., 2004). The aqueous and ethanolic extracts of garlic and ginger singly did not inhibit any of the test organisms. The highest inhibition zone of 19 mm was observed with a combination of extracts on *Staphylococcus aureus*. *Salmonella* spp. were resistant to almost all the extracts except lime (Munoz, *et al*., 2001).

Isolates of *Proteus* were found to be sensitive towards only basil oil while isolates of *Pseudomonas* and *E. coli* were found to be sensitive towards all oils used. *Pseudomonas* and *E. coli* were found to be most sensitive towards germanium oil. Isolates of *Klebsiella* were found to be most sensitive towards lemon grass oil and germanium oil (Reid, 1999). *Serratia* was observed to be most sensitive towards japanese mint oil. *Staphylococcus* was found to be most sensitive towards germanium oil.) Duman, *et al*. (2009) determined the antimicrobial activity of five essential oils namely, basil, chamomile, geranium, lemongrass and thuja against microorganisms isolated from patients having urinary tact infections (Johnson, 1991). The inhibitory effect was evaluated for antibiotic sensitive and resistant bacterial urinary isolates and yeast isolate (*Candida albicans*). Geranium oil exhibited antimicrobial activity against all the isolates, highest diameter of inhibition zone was observed against *Klebsiella pneumoniae* and *Staphylococcus aureus* isolates. The lowest values of minimum inhibitory concentrations were determined for geranium oil against *S. aureus* (8.96 mg mL^{-1}), *Proteus mirabilis* (17.92 mg mL^{-1}), *K. pneumoniae* (35.88 mg mL^{-1}) and *P. aeruginosa* (35.88 mg mL^{-1}). Geranium essential oil also exhibited a strong bactericidal activity against the uropathogens.

Conclusion

From the present study we concluded that the medicinal plants like *Punica granatum, Allium cepa and Stevia* have antimicrobial property against Urinary tract infections and uropathogens. Similarly the essential oils like Lemon grass oil, Basil oil and Japanese mint oil have antimicrobial activity against the Uropathogens. The bacterial isolates like *Pseudomonas*, *Proteus* , *Serratia* , *Staphylococcus* and *Klebsiella* were sensitive towards the aqueous and ethanolic extracts of essential oils and showed positive results towards medicinal plant extracts also. The isolates were more sensitive towards essential oils followed by medicinal plant extracts and antibiotics used and thus showing antimicrobial Susceptibility Pattern of Pathogenic Bacteria causing Urinary Tract Infection

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