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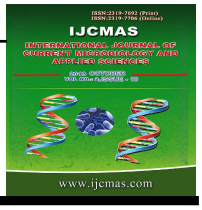

Original Research Article

Evaluation of Antibacterial Potential of Selected Plant Extracts on Bacterial Pathogens Isolated from Urinary Tract Infections

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Keywords

Urinary tract infection; antibacterial activity; phytochemicals; minimum inhibitory concentration.

A B S T R A C T

In the present study 200 urine samples were collected from both male and female patients suffering with urinary tract infections (UTI). A total of 75 bacterial cultures were isolated belonging to 5 species: *Escherichia coli* (44%); *Klebsiella pneumoniae* (25.33%); *Pseudomonas aeruginosa* (20%); *Enterobacter faecalis* (6.66%) and *Proteus mirabilis* (4%). Six plants (*Coriander sativum*, *Syzygium aromaticum*, *Cinnamomum cassia*, *Zingiber officinale*, *Terminalia chebula and Azadirachta indica*) and their parts (leaves, bark, flower, rhizome and fruit) were used to evaluate their antibacterial potential. Aqueous, methanolic and ethanolic extracts were used for studying antibacterial activity by agar well diffusion assay and Minimum inhibitory concentration method. Among the three extracts used, highest antibacterial activity was recorded with ethanolic extracts of *Cinnamomum cassia* on *E. coli* and least against *K. pneumoniae* with diameter of inhibition zones (DIZ) of 21.33 ± 0.57 and 15.66 ± 0.57 mm respectively. Preliminary phytochemical analysis of the plant parts revealed the presence of active compounds such as phenolics, tannins, alkaloids and flavonoids. The results obtained in this study clearly demonstrated higher and broad spectrum antibacterial activity of selected plant extracts on all five UTI isolates compared with ten standard antibiotics used for treating UTI.

Introduction

A urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. When it affects the lower urinary tract it is known as a simple cystitis (a bladder infection) and when it affects upper urinary tract it is known as pyelonephritis (a kidney infection). UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and they are the second most common cause of bacteremia in hospitalized patients (Samm and Norby, 2001). The annual cost to the

health care system of the United States attributable to community-acquired UTI alone is estimated to be approximately $ 1.6 billion (Foxman, 2002). UTIs are among the most common bacterial infections which are prevalent extraintestinal and affecting people of all ages from neonates to geriatric age group (Kunin, 1994). Worldwide, about 150 million people are diagnosed with UTI each year. It is estimated that about 35% of healthy women suffer with UTI infection at some stage in their life. About 5% of women each year suffer with the problem of painful urination (dysuria) and frequency. The incidence of UTI is greater in women than men, which may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors (Schaffer *et al*., 2001).

The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae. Members of this family include *E.coli*, *Klebsiella*, *Enterobacter* and *Proteus*. Also Gram positive *Staphylococcus* sp. plays a role in the infection (Kunin, 1997). *E.coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infections (Iroha, 2009). The frequency of *E.coli* in urine samples varies in different studies from 32% (Okada and Usui Abe, 1994), 40% (Nunezsanchez *et al*., 1999) and 75% (Goldstein, 2000).

Nowadays, drug resistance is a huge growing problem in treating infectious diseases like malaria, tuberculosis, diarrheal diseases, urinary tract infections etc. According to Goldman and Huskins (Goldman and Huskins, 1997) the improper and uncontrolled use of many antibiotics resulted in the occurrence of antimicrobial resistance, which became a major health problem worldwide. In the last 3 decades, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing UTIs (Tenver and McGowanJr, 1996; Kurutepe *et al*., 2005).

For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on earth to treat various infections, although only 1% have gained recognition by modern scientists (Kafaru, 1994). Owing to their popular use as remedies for many infectious diseases, search for plants containing antimicrobial substances is frequent (Betoni *et al*., 2006). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties (Lewis and Ausubel, 2006). A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their fewer side effects and reduced toxicity (Lee *et al*., 2007). There are several reports on the antimicrobial activity of different herbal extracts (Islam *et al*., 2008; de Boer *et al*., 2005). Many plants have been found to cure gastrointestinal disorders, respiratory diseases and cutaneous infections (Somchit *et al*., 2003; Santos *et al*., 1995). According to WHO, medicinal plants would be the best source for obtaining variety of drugs (Santos *et al*., 2005). These evidences contribute to support and quantify the importance of

screening natural products. Keeping in view the growing problem with UTIs and drug resistance, the present study was undertaken with an objective to find more efficient antibacterial agents of plant origin.

Materials and Methods

Collection of urine samples

A total of 200 urine samples from male and female patients admitted in hospitals due to UTI problems were collected from different hospitals and laboratories in Hyderabad, India. Guidelines for proper specimen collection were given to all patients on a printed card (Forbes BA *et al*., 2007). Before collecting a sample, the women were instructed to swab the vulvae and men to retract the foreskin and cleanse the glans penis. Mid stream urine was collected in sterile wide mouthed containers. Samples were transported to laboratory in an ice cold condition by adding boric acid at a final bacteriostatic concentration of 1.8% without delay (Porter and Bordie , 1969).

Colony count of urine samples

All urine samples were subjected to colony count by Urine Dip Slide method (VWR International BVBA, Leuven). The dip slide is immersed in urine sample so that both of the agars are completely covered by the sample; slide is removed from the sample, drained to remove any excess sample and incubated at 35 ± 2^0 C for 16 - 24 hrs. After incubation the dip slide is compared with the comparison chart provided. Equal or more than 10^4 CFU/ml of a single potential or two potential pathogens interpreted as positive UTI and a result of $10^2 – 10^4$ CFU/ml was repeated. A less than 10^2 CFU/ml was interpreted as negative UTI.

Isolation and identification of UTI bacterial pathogens

For isolation of UTI bacterial strains, loop full of urine samples were streaked on Mac Conkey agar, Blood agar and Nutrient agar plates (Hi Media, India & Merck, Germany) and incubated at 37 ± 2^0 C for 24 hrs. After incubation colonies were selected and characterized on the basis of morphological, cultural, physiological and biochemical characteristics (Mac Faddin, 2000). A presumptive identification was performed by Gram staining, oxidase activity, motility, catalase production, acid production in glucose, oxidation-fermentation (OF) test (glucose lactose and sucrose fermentation), Indole test, Voges-Proskauer test (VP) and hydrogen sulfide production. The bacterial isolates were identified with the help of Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984) and PIB computer kit (Bryan, 1993).

Plant material

A total of 6 plants and their parts: *Coriander sativum* (leaves), Clove *Syzygium aromaticum* (flower), *Cinnamomum cassia* (bark), *Zingiber officinale* (rhizome), *Terminalia chebula* (fruit), *Azadirachta indica* (fruit) were collected based on ethnomedical importance from different areas in and around Hyderabad, (A.P), India. All specimens were identified by Prof. Dr. S. Varalaxmi, Head Dept. of Botany, Govt. City College, Hyderabad, India. The voucher specimens have been maintained in Department of Microbiology, Mumtaz Degree and P.G College, Hyderabad, India.

Preparation of extracts

All plant parts were washed with distilled water dried in shade, grinded to fine powder and stored in airtight containers at room temperature in dark until used. The powdered samples were subjected to extraction by the following method of Gupta *et al*. (2009).

Aqueous extraction

For aqueous extraction 10g of air dried powder was mixed well in 100ml distilled water with constant stirring for 30 minutes. The solution was kept at room temperature for at least 24h and then filtered using muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 minutes. The supernatant was again filtered using Whattman's Filter No. 1 under strict aseptic conditions. The filtrate was collected in fresh sterilized glass tubes and stored at 4°C until use. Aqueous extract was prepared in final concentration of 100 mg/ml.

Extraction using Organic Solvents

10g of air dried powder was thoroughly mixed with 100ml organic solvent (ethanol and methanol). The mixtures thus obtained were filtered through muslin cloth and then re-filtered by passing through Whattman's filter No. 1. The filtrates were then concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solutions of crude extracts were prepared by mixing well the appropriate amount of dried extracts with appropriate solvent to obtain a final concentration of 100 mg/ml. Each solution was stored at 4°C after collecting in sterilized glass tubes until use.

Antibacterial Susceptibility Assay

Agar well diffusion assay (Perez *et al*., 1990) was the key process used to evaluate the antibacterial potential of plant extracts. Extracts were first sterilized by sterile membrane syringe filter (pore size 0.45 μm, manufactured by Pall Life Sciences). Petri dishes (100mm) containing 18ml of Mueller Hinton Agar were seeded with approximately 100μl inoculum of bacterial strain (inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 CFU/ml). Media was allowed to solidify. Wells of 6mm diameter were cut into solidified agar media using a sterilized cup-borer. 100μl of each extract was poured in the respective well and the plates were incubated at 37°C overnight. The experiment was performed in triplicate under strict aseptic conditions to ensure consistency of all findings. The antibacterial activity of each extract was expressed in terms of the mean of diameter of inhibition zone (DIZ) in mm ± SD, produced by each extract at the end of incubation period. Organic solvents used in preparation of extracts were also used as negative controls during the study. Ten (10) commercially available standard antibiotics (ampicillin**,** ciprofloxacin, gentamycin, norfloxacin, nitrofurantoin, nalidixic acid, trimethoprim-sulpha methoxazole, clotrimazole, cefotaxime, and tetracycline) were also used in the present study for testing the susceptibility of isolated UTI pathogens.

Assessment of Minimum Inhibitory Concentration

MIC (minimum inhibitory concentration) of active extracts thus obtained was further examined by standard two-fold microdilution broth methodology (NCCLS, USA, 1998). A stock solution of

each active extract was serially diluted in 96-wells microtiter plate with Mueller Hinton broth to obtain a concentration of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 mg/ml. A standardized inoculum for each bacterial strain was prepared so as to give an inoculum size of approximately 5 x 10^5 CFU/ml in each well. Microtiter plates were then kept at 37°C for an overnight incubation. Following incubation, the MIC was calculated as the lowest concentration of the extract inhibiting the visible growth of bacterial strain using reflective viewer.

Phytochemical Analysis

Phytochemical analysis of the extracts was carried out by using methods as described by Harborne (1998) and Kolkate *et al*. (2003). By this analysis, the presence of several phytochemicals like phenolics, alkaloids, flavonoids, tannins, saponins, steroids and glycosides were tested.

Statistical Analysis

Results obtained were analyzed statistically and values were expressed as Mean ± SD.

Result and Discussion

In the present study 150 patients out of 200 were shown to be urine culture positive as their colony count was equal or more than 10^4. The demographic characterization of the patients showed that, the majority of them (80%) were living in urban area. Significant proportion were females (73.3%) , 90 in the age group of 19 to 39 years (60%), 115 married (76.6%) and 72 were either illiterate or capable of read and write (48%). The age range of the patients was between 15-60 years (Table-1). Seventy bacterial isolates were isolated from 150 urine samples. The isolates were characterized and identified by studying different properties as mentioned in materials and methods. The identification characteristics were cross checked with those of standard manuals (Mac Faddin FJ 2000, Kreig RN & Holt GJ 1984 and Bryan TN 1993). The biochemical characteristics revealed that, these isolates belong to 5 species (Table-2). Of these *Escherichia coli* is the predominant one (44%); *Klebsiella pneumoniae* (25.33%); *Pseudomonas aeruginosa* (20%); *Enterobacter faecalis* (6.66%) and *Proteus mirabilis* (4%) as depicted in Figure-1.

Based on the results obtained from susceptibility testing it was observed that all the bacteria isolated from UTI showed highest degree of resistance to gentamycin, nalidixic acid, trimethoprim-sulphamethaoxazole, clotrimazole and cefotaxime which are commonly prescribed drugs for UTI treatment (Table-3). The antibiotics which were effective up to some extent were ampicillin, norfloxacin and tetracycline.

Results obtained for antibacterial studies reveal following findings. Aqueous, ethanolic and methanolic extracts of plants exhibited antibacterial activity towards all five isolated UTI pathogens, with more activity observed with ethanolic extracts. There was significant variation in the antibacterial activities (DIZ values) of different plant extracts. The aqueous extracts have shown moderate antibacterial effect on isolated UTI pathogens. High antibacterial activity was recorded for *Zingiber officinale* and *Syzygium aromaticum* compare to other plants with DIZ values in range of 5.7 ± 0.60 and 11.6 ± 0.57 mm and 3.67 ± 0.58 and 11.0 ± 0.60 mm respectively. The aqueous extracts of other plants *Coriander*

sativum, Cinnamomum cassia and *Azadirachta indica* have shown less antibacterial activity (Table-4).

The methanolic extracts of all the plants have shown good antibacterial effect against the UTI isolates (Table-5). The most effective antibacterial activity was recorded for *Cinnamomum cassia* which has inhibited all 5 UTI isolates. The maximum effect was observed against *E. coli* (DIZ value 19.66 ± 0.57 mm) and least against *K. pneumoniae* (DIZ value 10.0 ± 0.81 mm). *P. aeruginosa* and *P. mirabilis* were equally inhibited with a DIZ value of 18 ± 0.60 and 18.6 ± 0.57 mm and *E. faecalis* with 16 ± 0.60 mm respectively. Second highest antibacterial activity was observed for *Syzygiym aromaticum* with a DIZ range between 8.33 ± 0.57 and 19.33± 0.57 mm. The highest antibacterial effect was recorded for *E. coli* (19.33 ± 0.57mm), followed by *P. aeruginosa* (17.66±0.57mm), *P. mirabilis* (15.7 ± 0.58mm), *E. faecalis* (13.66±0.57mm) and least for *K. pneumoniae* (8.33 ± 0.57 mm). Among all the methanolic plant extracts, lowest antibacterial activity was recorded for *Coriander sativum* which has shown DIZ value of 11.66 ± 0.57 mm against *P. aeruginosa* and lowest value of 4.66 ± 0.57 mm against *P. mirabilis*.

In the present study highest antibacterial activity was exhibited by ethanolic plant extracts. Among all plant extracts highest DIZ values were recorded for *Cinnamomum cassia* in the range of 21.33 ± 0.57 and 15.66 ± 0.57 mm against UTI bacterial isolates (Table-6). Highest antibacterial effect was observed against *E. coli* (21.33 ± 0.57 mm), followed by *P. mirabilis* (20.33 ± 0.57 mm), *P. aeruginosa* (19.66 ± 0.57 mm), *E. faecalis* (17.66 ± 0.57 mm) and least for *K. pneumoniae* (15.66 ± 0.57 mm). The pattern of antibacterial activity shown by other ethanolic plant extracts was similar to that of methanolic extracts where in, next higher activity was observed for *Syzygium aromaticum*, *Zingiber officinale, Azadirachta indica* and *Coriander sativum* but with higher DIZ values than obtained for methanolic extracts. Among ethanolic extracts lowest antibacterial effect was observed for *Coriander sativum* with a DIZ values in range of 6.33 ± 0.57 to 14.66 ± 0.57 mm.

The phytochemical studies reveal that flavonoids, phenolics, alkaloids and tannins are present in all these selected plants. Steroids are present in all plants except *Zingiber officinale*. Results of other phytochemical constituents are shown in Table-7. Quantitative evaluation of antibacterial activity (MIC) was carried out by microdilution method for methanolic and ethanolic extracts. Four plants out of six were used for studying MIC, as two plants (*Terminalia chebula* & *Coriander sativum*) revealed very low antibacterial effect by agar well diffusion method. Figure-2 shows the MIC of selected plant extracts: *Cinnamomum cassia, Syzygium aromaticum, Azadirachta indica* and *Terminalia chebula* on five bacterial UTI isolates. A wide range of MIC values were recorded depending on the microbial strain.

Urinary tract infection is a complicated problem that continues to present new challenges due to change in the etiology of UTI and the antimicrobial resistance of urinary pathogens over the years. Factors such as the changing in patient population and extensive use and abuse of antimicrobial agents could contribute to changes in the microbial profile of urinary tract isolates (Mady and Helmi, 2003).

Table.1 Characteristics of Urine culture positive patients (Hyderabad, India).

Variables	Number	Percentage
Location		
Urban	120	80.0
Rural	30	20.0
Sex		
Male	40	26.6
Female	110	73.3
Age Categories		
Less or equal to 18 years	20	13.3
19 to 39 years	90	60.0
40 to 60 years	40	26.6
Education level		
Illiterate	30	20.0
Write and read only	42	28.0
Up to 8 grade	20	13.3
Up to 12 grade	35	23.3
University level	23	15.3
Marital status		
Single	35	23.3
Married	115	76.6

Table.2 Biochemical characteristics of UTI bacterial isolates

S.No	Grams Nature	TSI		Man	Mot	In	MR	VP	Cit	Ur	Oxi	Cat	H_2S	Identity of isolates
		Slant	Butt											
1	-	-	-	Acid	+	+	+	-	-	-	-	-	-	*E. coli*
2	-	+	+	Acid	-	-	-	+	+	+	-	-	-	*K. pneumoniae*
3	-	-	+	Acid	+	-	+	-	-	+	+	+	-	*P. aeruginosa*
4	-	+	-	Acid	+	-	-	+	+	-	-	-	-	*E. faecalis*
5	-	+	+	-	+s	-	-	-	+	+	-	-	+	*P. mirabilis*

TSI- Triple sugar iron; Man-mannitol; Mot- motility; In-indole; MR-methyl red; VP-voges proskaeur; Cit-citrate; Ur-urease; Oxi-oxidase; Cat- catalase; H_2S-hydrogen sulphide; '+':positive '-':negative 's':swarming motility.

Table.3 Antibacterial susceptibility of isolated bacterial UTI pathogens

Standard Antibiotics	Name of the bacterial isolates				
	E. coli	***K.pneumoniae***	***P.aeruginosa***	***E.faecalis***	***P.mirabilis***
Ampicillin	R	S	S	S	R
Ciprofloxacin	R	R	S	S	R
Gentamycin	R	R	R	R	R
Norfloxacin	S	S	S	R	S
Nitrofurantoin	R	R	S	R	S
Nalidixic acid	R	R	R	R	R
Trimethoprim-sulphamethaoxazole (SXT)	R	R	R	R	R
Clotrimazole	R	R	R	R	R
Cefotaxime	R	R	R	R	R
Tetracycline	S	R	S	S	S

R- Resistant S- Sensitive

Table.4 Antibacterial activity of Aqueous Plant Extracts on Bacterial UTI Isolates

AQUEOUS PLANT EXTRACTS	**Diameter of Inhibition Zone (DIZ) in mm**				
	E. coli	***K.pneumoniae***	***P.aeruginosa***	***E.faecalis***	***P.mirabilis***
Zingiber officinale (rhizome)	11.66±0.57	7.67±0.58	5.70±0.60	9.67±0.58	8.33±0.58
Syzygium aromaticum (flower)	6.30±0.60	3.67±0.58	11.0±0.60	5.70±0.60	6.67±0.58
Azadirachta indica (fruit)	10.0±1.0	3.67±0.58	4.33±0.60	5.67±0.58	2.66±0.57
Terminalia chebula (fruit)	8.33±0.57	4.66±0.57	4.66±0.57	2.66±0.57	2.66±0.57
Coriander sativum (leaf)	3.66±0.57	5.66±0.57	7.66±0.57	5.66±0.57	2.66±0.57
Cinnamomum cassia (bark)	4.66±0.57	2.66±0.57	3.66±0.57	5.66±0.57	7.0±1.0

Table.5 Antibacterial activity of Methanolic Plant Extracts on Bacterial UTI Isolates

Methanolic Plant Extracts	**Diameter of Inhibition Zone (DIZ) in mm**				
	E. coli	***K.pneumoniae***	***P.aeruginosa***	***E.faecalis***	***P.mirabilis***
Cinnamomum cassia (bark)	19.66±0.57	10.0±0.81	18.0±0.60	16.0±0.6	18.6±0.57
Syzygium aromaticum (flower)	19.33±0.57	8.33±0.57	17.66±0.57	13.66±0.57	15.7±0.58
Zingiber officinale (rhizome)	17.0±0.60	7.67±0.58	16.0±0.60	9.67±0.58	11.66±0.57
Azadirachta indica (fruit)	15.66±0.57	5.66±0.57	11.66±0.57	11.66±0.57	7.66±0.57
Terminalia chebula (fruit)	13.66±0.57	9.33±0.57	9.66±0.57	5.66±0.57	4.66±0.57
Coriander sativum (leaf)	8.66±0.57	4.66±0.57	11.66±0.57	5.66±0.57	4.66±0.57

Table.6 Antibacterial activity of Ethanolic Plant Extracts on Bacterial UTI Isolates

Ethanolic Plant Extracts	**Diameter of Inhibition Zone (DIZ) in mm**				
	E. coli	***K.pneumoniae***	***P.aeruginosa***	***E.faecalis***	***P.mirabilis***
Cinnamomum cassia (bark)	21.33±0.57	15.66±0.57	19.66±0.57	17.66±0.57	20.33±0.57
Syzygium aromaticum (flower)	19.66±0.57	13.66±0.57	17.66±0.57	18.33±0.57	18.7±0.58
Zingiber officinale (rhizome)	20.0±0.60	9.0±1.0	17.0±0.60	15.7±0.58	14.33±0.57
Azadirachta indica (fruit)	18.66±0.57	9.33±0.57	14.7±0.58	7.66±0.57	18.33±0.57
Terminalia chebula (fruit)	17.66±0.57	7.33±0.57	14.33±0.57	7.66±0.57	18.33±0.57
Coriander sativum (leaf)	11.66±0.57	6.33±0.57	9.66±0.57	7.33±0.57	14.66±0.57

Table.7 Phytochemical Analysis of Selected Plants

PLANT EXTRACTS	TAN	ALK	FLAV	SAP	GLY	STER	PHEN
Coriander sativum (leaf)	+	+	+	+	+	+	+
Syzygium aromaticum (flower)	+	+	+	+	+	+	+
Cinnamomum cassia (bark)	+	+	+	-	+	+	+
Zingiber officinale (rhizome)	+	+	+	+	+	-	+
Terminalia chebula (fruit)	+	+	+	+	+	+	+
Azadirachta indica (fruit)	+	+	+	+	+	+	+

TAN: Tannins; ALK: Alkaloids; FLAV: Flavonoids; SAP: Saponins; GLY: Glycosides; STER: Steroids; PHEN: Phenolics "+": Presence "-": Absence

Figure.1 Pathogenic Bacteria isolated from urinary tract infections

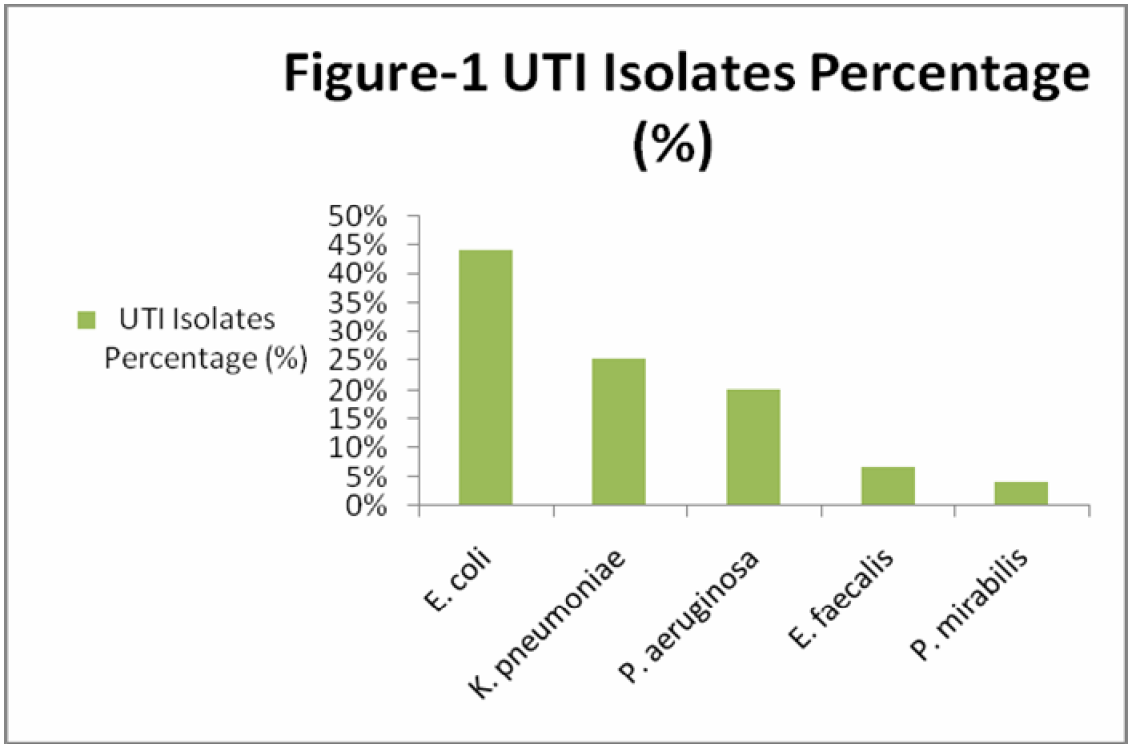
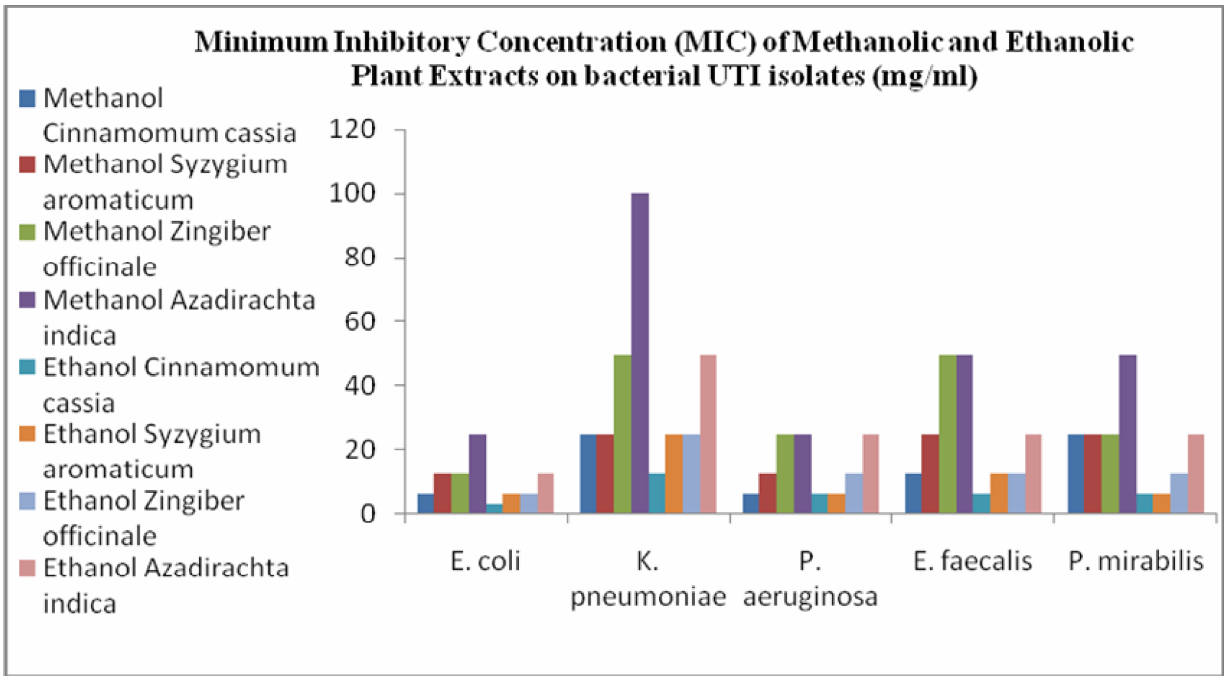


Figure.2 Minimum Inhibitory Concentration (MIC) by Microdilution method (mg/ml).



The emergence of drug resistance with patient's poor compliance, drugs adverse effects and the higher cost of therapy combinations, indicates a strong need for a therapy regimens with similar or higher antibiotics beneficial properties but with better adverse effects profiles. Results of the current study suggest a class effect antibacterial activity for clinical isolates, and indicate the superiority of the antibacterial activity of plant extracts compared to standard antibiotics.

Antibacterial activity of aqueous, methanolic and ethanolic extracts of six plants: *Coriander sativum* (leaves), Clove *Syzygium aromaticum* (flower), *Cinnamomum cassia* (bark), *Zingiber officinale* (rhizome), *Terminalia chebula* (fruit), *Azadirachta indica* (fruit) was tested on five bacterial clinical isolates: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter faecalis* and *Proteus mirabilis* from patients suffering with UTI. Similar UTI pathogens have been reported by Amit Kumar *et al*., (2012), Fuad M. M. H et al. (2012) and Al-Jiffri O et al. (2011). The antibacterial potency was initially determined by the agar well diffusion method (as shown in Table-4, 5 and 6) followed by quantitative evaluation of antibacterial activity by MIC method (as shown in Figure-2).

Ethanolic extracts of all the plants exhibited higher antibacterial effect than aqueous and methanolic extracts. Among all ethanolic extracts, *Cinnamomum cassia*-bark exhibited highest antibacterial activity which inhibited all five bacterial UTI isolates in following order- *E. coli* > *P. mirabilis* > *P. aeruginosa* > *E. faecalis* > *K. pneumoniae*. Antibacterial effect of *C. cassia* bark extracts was studied by Anjana Sharma *et al*., (2009) where in high activity was recorded for ethanolic extract against *P. aeruginosa* of UTI origin with a DIZ of 16mm. These findings and the results obtained in our

study clearly confirm the effectiveness of *C. cassia* bark extracts on inhibition of bacterial activity.

After *Cinnamomum cassia*, strong antibacterial effect was recorded for ethanolic extracts of *Syzygium aromaticum*- flower. The order of inhibition followed same pattern exhibited by *C. cassia* except for *E. faecalis* and *P. mirabilis* which were equally inhibited. In an interesting study on *E.* coli isolated from UTI samples, the antibacterial effect of cold water, boiling water and ethanolic extracts of *Syzygium aromaticum*-flower was studied by Al-Jiffri *et al*., (2011). In this study high antibacterial activity was recorded for ethanolic extracts with a zone of 18 mm, thereby again giving a confirmation that ethanolic extracts exert potential antibacterial effect.

Antimicrobial activity of *Syzygium aromaticum* was also studied by Rahman *et al*., (2011) on five bacterial strains with DIZ values range between 7.33 and 7.83 mm. They have reported high antibacterial activity in acetone extracts rather than ethanolic extracts. The DIZ values obtained in our study were higher than all these previous reports which clearly show that *Syzygium aromaticum* has strong antibacterial activity.

The antibacterial activity of ethanolic extracts of *Zingiber officinale* – rhizome was more against *E. coli* and least against *K. pneumoniae*, with slight variation in pattern of antibacterial activity exhibited compared to *Cinnamomum cassia* and *Syzygium aromaticum.* Following order of antibacterial activity was observed: *E. coli* > *P. aeruginosa* > *E. faecalis* > *P. mirabilis* >*K. pneumoniae*. The antibacterial effectiveness of ethanolic extracts on UTI pathogens was also reported by Anjana Sharma *et al*., (2009). Among water, acetone and ethanol extracts, highest antibacterial effect was recorded for ethanolic extracts. The order of antibacterial activity observed was: *P. aeruginosa* > *E. coli* > *K. Pneumoniae*. There was no inhibitory effect observed for all the three extracts against *P. mirabilis* and *E. faecalis*. The results obtained in our study are superior to these previous reports.

In our study *Azadirachta indica* and *Terminalia chebula* fruit extracts expressed similar antibacterial effect against the tested UTI bacterial isolates. As observed with other plant extracts higher activity was recorded for ethanolic extracts. The highest antibacterial effect was observed against *E. coli*. The antibacterial effect of *Azadirachta indica* was also studied by Yerima *et al*., (2012) and Dhanya Kumar *et al*., (2011). Interestingly in both the reports antibacterial activity was observed only at higher concentrations of *Azadirachta indica* extracts however, at lower concentrations all tested bacteria were resistant.

In comparison all extracts (aqueous, methanolic and ethanolic) used in our study expressed good antibacterial effect against all the tested bacteria. Hogade *et al.*, (2011) studied the antibacterial activity of aqueous fruit extracts of *Terminalia chebula* on gram positive and gram negative bacteria. *E. coli* is the most affected organism in their study followed by *K. pneumoniae* and *P. aeruginosa*. Broad spectrum antibacterial activity of *Terminalia chebula* fruit extracts was reported on selected gram positive and gram negative bacteria by Kannan *et al*., (2009). In their results ethanolic extracts expressed strong inhibitory effect against

E. coli, *P. aeruginosa* and *K. pneumoniae*. In the present study ethanolic extracts of *Coriander sativum* expressed less antibacterial effect when compared with other plant extracts. Among five UTI isolates, higher effect was observed against *P. mirabilis* and least against *K. pneumoniae*. Ates and Erdogrul (2003) studied the antibacterial effect of ethanol, ethyl acetate, acetone and chloroform extracts of *Coriander sativum*. Interestingly none of the extract expressed any antibacterial effect. Dash *et al*., (2011) reported antibacterial activities of methanol and acetone extracts of *Coriander sativum* on selected bacteria. Methanol extracts expressed high antibacterial activity against *P. aeruginosa* and *E. coli*. The DIZ values obtained in our study are much higher than all these previous reports.

The results obtained for the plant extracts in the present study showed significant antibacterial activity against the five pathogenic UTI isolates tested. Among all the tested bacteria *K. pneumoniae* is the least affected organism giving a clue that capsule and other determinant factors such as enzyme may be responsible for decreasing the effectiveness of the components present in plant extracts. MIC by micro dilution showed good results compared to well diffusion method as there may be a problem with the diffusion of the biological component into the agar. The hydrocarbon components either remain on the surface of the medium or evaporate (Griffin *et al*., 2000). That could be the reason for the better results obtained by the microdilution method. Broth method has advantage of lower workloads for a large number of replicates and the use of small volumes of the test substance and growth medium. (Sokovic *et al*., 2007). The difference in the antibacterial activity with the same source when extracted with different solvent has proven that not all phytochemicals that are responsible for antibacterial activity are soluble in a single solvent. Hence solvents of different polarity should be employed as discussed in this study (polar and nonpolar).

In the present study ethanolic extracts of selected plants expressed highest and broad spectrum antibacterial activity to pathogenic UTI isolates. Two possibilities that may account for this higher antibacterial activity of ethanolic extracts are the nature of biological active components (alkaloids, flavonoids, essential oils, terpenoids, tannins etc.), which may be enhanced in the presence of ethanol; and stronger extraction capacity of ethanol that may have yielded a greater number of active constituents responsible for antibacterial activity (Gosh *et* al., 2008).

Based on the results obtained we can conclude that all selected plants used in this study expressed broad spectrum antibacterial activity on bacterial UTI isolates with highest activity recorded for ethanolic extracts. The data obtained for antibacterial activity of 10 standard antibiotics commonly used for UTI treatment concludes that, most of the antibiotics were ineffective in inhibiting the growth of these bacterial isolates, on the other hand all the extracts- aqueous, methanolic and ethanolic exerted good antibacterial activity. Importantly the results suggest that these plants contain active ingredients which qualify them for medicinal use. The presence of phytocompounds in the extracts including phenols, tannins and flavonoids as major constituents may be responsible for the antibacterial activity.

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