JCBPS; Section B; May 2017 – July 2017, Vol. 7, No. 3; 718-732, E- ISSN: 2249 –1929

[DOI: https:/doi.org/10.24214/jcbps.B.7.3.71832]

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT Research Article

Antibacterial activity of Herbal and Spice Extracts on Bacterial Clinical Isolates of Urinary Tract Infections

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Received: 20 April 2017**; Revised:** 29 April 2017; **Accepted:** 06 May 2017

Abstract: In the present study 370 urine samples were collected from two diagnostic laboratories of Hyderabad, Telangana State, India, of which 244 urine samples were tested as UTI positive and 116 bacterial cultures were isolated belonging to 5 species: *Escherichia coli* (48); *Klebsiella pneumoniae* (30); *Pseudomonas aeruginosa* (24); *Enterococcus faecalis* (8) and *Proteus mirabilis* (6). Three selected plants (*Cinnamomum cassia, Syzygium aromaticum* and *Azadirachta indica*) and their parts (bark, flower, and leaf) were used to test their antibacterial activity by agar well diffusion assay and MIC was determined. Three extracts- aqueous, ethanolic and methanolic were tested; highest antibacterial activity was observed with ethanolic extracts of *Cinnamomum cassia* with strong effect on *E. coli* and lowest against *K. pneumoniae* with diameter of inhibition zones (DIZ) of 20.56 ± 0.40 and 15.66 ± 0.57 mm respectively. Preliminary phytochemical analysis of the plant parts revealed the presence of active compounds such as flavonoids, phenolics, tannins and alkaloids. The results obtained in this study revealed broad spectrum antibacterial activity of selected plant extracts on all five UTI bacterial isolates compared with standard antibiotics used for UTI treatment.

Keywords: Urinary tract infection, antibacterial activity, phytochemicals, minimum inhibitory concentration.

INTRODUCTION

The most common extra intestinal infections are urinary tract infections (UTIs) which affect people of all age groups[1]. Globally about 150 million people are diagnosed with UTI every year[2]. The prevalence of UTI is higher in women due to following factors- a) urothelial mucosal adherence to the mucopolysaccharide, b) anatomical predisposition and c) other host factors[3]. UTI is a bacterial infection, which can be simple cystitis (bladder infection) or pyelonephritis (kidney infection). UTI has become the most common hospital-acquired infection, accounting for 35% of nosocomial infections, and **is** the second most common cause of bacteremia in hospitalized patients[4]. The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae. *Escherichia coli* is the most common causative agent with 75-90% causes of UTI infection [5-7]. The other gram negative bacteria causing UTI are *Klebsiella* spp., *Proteus mirabilis* and *Pseudomonas aeruginosa*. The gram positive bacteria that commonly causes UTI are Enterococci and coagulase negative Staphylococci[8].

Nowadays, drug resistance is a huge growing problem in treating infectious diseases like malaria, tuberculosis, diarrheal diseases, urinary tract infections etc. The improper and uncontrolled use of many antibiotics resulted in the occurrence of antimicrobial resistance, which became a major health problem worldwide[9]. 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 [10,11]. Another cause of drug resistance in pathogenic microorganisms is genetic due to horizontal gene transfer via plasmids, transposons and bacteriophages; recombination of foreign DNA in bacterial chromosome and mutations in chromosomal segments[12]. In the past decades many drug resistant bacteria have been discovered, some of which are methecillin resistant *Staphylococcus aureus* (MRSA)[13], *Serratia marcescens*[14], multi-drug resistant *Pseudomonas aeruginosa*[15], vancomycin resistant Enterococci (VRE)[16] and beta lactamase (ESBL) resistant Enterococci[17].The matter of drug resistance is a serious public health issue mainly in developing countries where high level of poverty, poor hygienic conditions as well as fake and adulterated drugs or imitated drugs are in the circulation of medical practices[18]. Hence, the changing susceptibility patterns of microorganisms causing UTI should be taken into account with urgency and antimicrobial susceptibility testing of these pathogens should be conducted on regular basis and in various regions.

For thousands of years in various parts of the world natural products have been used in traditional medicine before the discovery of antibiotics and other modern drugs. The remarkable antimicrobial property of some plants in treating diseases has been beyond belief. According to one of the study 10% of all flowering plants on earth have been used by local communities to treat various infections, whereas only 1% has gained recognition by modern scientists[19]. According to WHO, medicinal plants would be the best source for obtaining variety of drugs[20]. 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[21]. Search for plants containing antimicrobial substances is more common due to their popular use as remedies for many infectious diseases [22] and also due to their fewer side effects and reduced toxicity [23]. There are several reports on the antimicrobial activity of different herbal extracts[24, 25]. Many plants have been found to cure gastrointestinal disorders, respiratory diseases and cutaneous infections[26, 27]. These evidences contribute to support and quantify the importance of screening natural products. Keeping in view the growing problem with UTIs and increase in antimicrobial resistance, the present study was carried out with an objective to search for more efficient antibacterial agents of plant origin.

MATERIALS & METHODS

Sample Collection: A total of 370 urine samples were collected from twodiagnostic laboratories of Hyderabad, Telangana State, India. Prior to sample collection patients were instructed to collect midstream urine samples. All samples were collected in sterile containers and were transported to laboratory in an ice cold condition by adding boric acid at a final bacteriostatic concentration[28] of 1.8% .

Screening of Urine Samples: Urine samples were screened for positive or negative UTI by Urine Dip Slide method (Thermo Fisher Scientific). The clinical dip slide has a coated culture media Mac Conkeys agar on one side and CLED agar on the other. 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 and drained to remove any excess urine and incubated at 37^0 C for 24 hrs. After incubation the dip slide is compared with the comparison chart provided. Equal or more than 10^4 CFU/ml was interpreted as positive UTI and a less than 10^2 CFU/ml was interpreted as negative UTI. A result of 10^2 –10^4 CFU/ml was repeated.

Isolation and Identification of Bacteria from Urine Samples: The urine samples which were identified as positive for UTI were further subjected to isolation of bacteria. Streak plate method was used for isolation of pure cultures for which loop full of urine samples were streaked on Mac Conkey agar, Blood agar and Nutrient agar plates (Hi Media, India) and incubated at 37^0 C for 24 hrs. After incubation colonies were selected and characterized on the basis of morphological, cultural, physiological and biochemical characteristics[29]. 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[30].

Collection of Plant material:A total of 3 plants and their parts: *Cinnamomum cassia* (bark), *Syzygium aromaticum* (flower), and *Azadirachta indica* (leaf) were collected from local market in Hyderabad, Telangana, India. All specimens were identified in Dept. of Botany, Mumtaz College, and voucher specimens **(Voucher Specimen No: MCBDA/C/S-20/06/13-16)** have been maintained in Department of Microbiology, Mumtaz Degree and P.G College, Hyderabad, India.

Preparation of Extracts: The plant parts were washed with tap water followed by 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 aqueous and organic solvent extraction by the method of Gupta *et al.* [31].

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 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. 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 of each methanol and ethanol. 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 Assay: Agar well diffusion assay[32] was used to test the antibacterial activity of plant extracts. Petri dishes (100mm) containing 18ml of Mueller Hinton Agar were seeded with approximately 100µl inoculum of each bacterial strain (inoculum size was adjusted 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 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 commercially available standard antibiotics were also used in the present study for testing the resistance/sensitivity of isolated UTI pathogens.

Determination of Minimum Inhibitory Concentration: Minimum inhibitory concentration (MIC) of active extracts (methanolic and ethanolic) was determined by standard two-fold microdilution broth methodology[33]. 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 inoculums for each bacterial isolate was prepared 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 bacteria.

Phytochemical Analysis: Phytochemical analysis of the extracts was carried out by the methods of Harborne[34] and Kolkate*et al.*[35].Presence of several phenolics, alkaloids, flavonoids, tannins, saponins, steroids and glycosides were tested by this analysis.

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

RESULTS

In the present study 244 urine samples were screened as positive for UTI based on results obtained by dip slide method.116 bacterial cultures were isolated; the isolates were characterized and identified by studying different characteristics as mentioned in materials and methods. The identification characteristics were cross checked with those of standard manuals [29, 30]. The identification results revealed that, these isolates belong to 5 species **(Table-1).**

Table1: Identification of UTI Bacterial isolates by Cultural and Biochemical Characteristics

Probable Identityof isolates	Grams Nature	TSI		Man	Mot	In	MR	VP	Cit	Ur	Oxi	Cat	H_2S
		Slant	Butt										
E. coli	-	-	-	Acid	+	+	+	-	-	-	-	-	-
K. pneumoniae	-	+	+	Acid	-	-	-	+	+	+	-	-	-
P. aeruginosa	-	-	+	Acid	+	-	+	-	-	+	+	+	-
E. faecalis	-	+	-	Acid	+	-	-	+	+	-	-	-	-
P. mirabilis	-	+	+	-	+s	-	-	-	+	+	-	-	+

TSI- Triple sugar iron test; 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.

Among the isolated bacteria*Escherichia coli*was the predominant one (41.37%) followed by *Klebsiella pneumoniae* (25.86%); *Pseudomonas aeruginosa* (20.68%); *Enterococcus faecalis* (6.89%) and *Proteus mirabilis* (5.17%) as depicted in **Figure-1**.

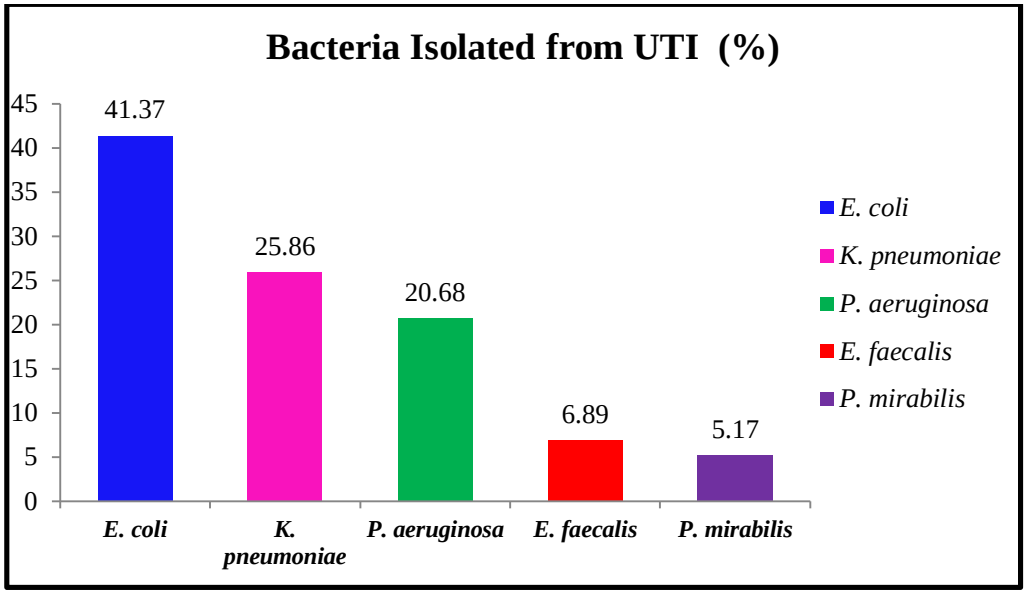

Figure 1: Details of Bacteria isolated from **UTI** positive urine samples

Table2: Antibacterial resistance/sensitivity of isolated bacterial UTI pathogens

Standard Antibiotics	**Bacterial Isolates of UTI**				
	E. coli	***K.pneumoniae***	***P.aeruginosa***	***E.faecalis***	***P.mirabilis***
Ampicillin	+	-	-	-	+
Cefotaxime	+	+	+	+	+
Ciprofloxacin	+	+	-	-	+
Clotrimazole	+	+	+	+	+
Gentamycin	+	+	+	+	+
Nalidixic acid	+	+	+	+	+
Nitrofurantoin	+	+	-	+	-
Norfloxacin	-	-	-	+	-
Tetracycline	+	+	-	-	-
Trimethoprim-sulphamethaoxazole	+	+	+	+	+

+Resistant – Sensitive

Based on the results obtained from resistance/susceptibility testing it was observed that the bacterial isolates showed highest degree of resistance to gentamycin, nalidixic acid, trimethoprim-sulphamethaoxazole, clotrimazole and cefotaxime which are commonly prescribed antibiotics for UTI treatment (Table-2). 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 *Azadircahta indica* compare to other two plants with DIZ values in range of 2.66 ± 0.57 to 8.66 ± 0.41 mm respectively with strong effect on *E.coli* and least on *P. mirabilis*. The aqueous extracts of other plants *Cinnamomum cassia* and *Szygyium aromaticum* have shown less antibacterial activity (Figure-2).

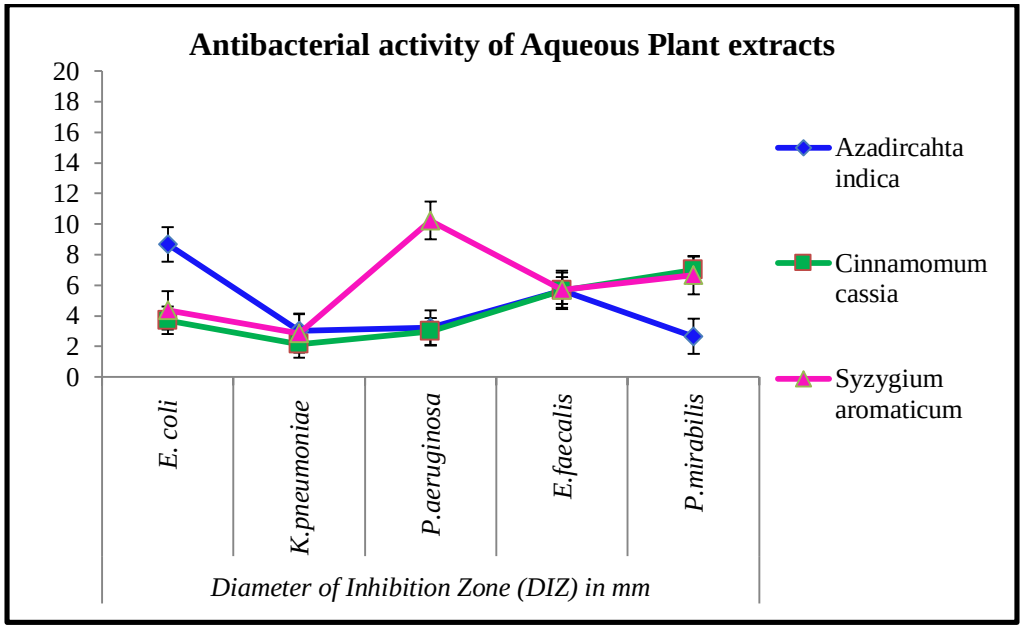

Figure 2: Antibacterial Activity of Aqueous plant extracts on UTI Bacterial isolates

The methanolic extracts of all the plants have shown good antibacterial effect against the UTI isolates (Figure-3). 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 ± 0.10 mm). *P. aeruginosa* and *P. mirabilis* were more or less similarly inhibited with a DIZ value of 18.03 ± 0.05 and 18.66 ± 0.57 mm and *E. faecalis* with 14.86 ± 0.15 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 ± 0.57 mm), *P. mirabilis* (15.70 ± 0.51 mm), *E. faecalis* (12.60 ± 0.20 mm) and least for *K. pneumoniae* (8.33 ± 0.57 mm). Comparatively *Azadirachta*

indica extracts showed low antibacterial activity than the other two plant extracts. The DIZ values range between 5.66± 0.57 and 15.66± 0.57 mm.

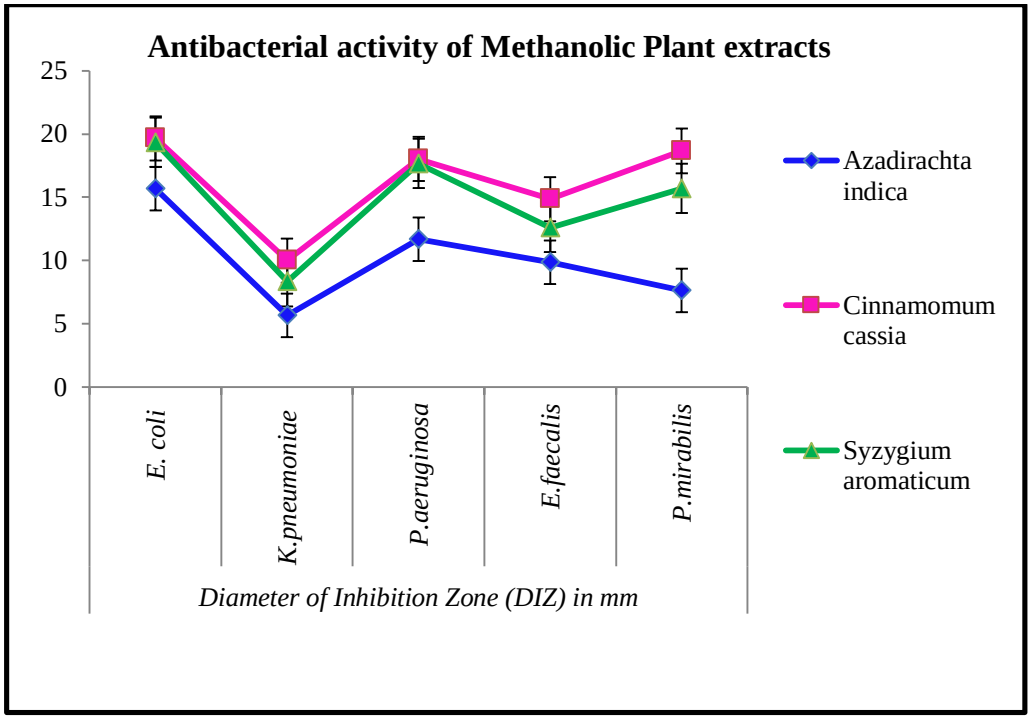

Figure 3: Antibacterial Activity of Methanolic plant extracts on UTI Bacterial isolates

In the present study highest antibacterial activity was expressed by ethanolic plant extracts. Among the three selected 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 (Figure-4). 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). After *Cinnamomum cassia* next highest antibacterial activity was observed for *Syzygium aromaticum* with DIZ value in range of 13.66±0.57 and 18.70±0.43 mm. Among ethanolic extracts lowest antibacterial effect was observed for *Azadircahta indica* with a DIZ values in range of 9.33±0.57 to 17.86±0.15 mm.

The phytochemical studies reveal that flavonoids, phenolics, alkaloids and tannins are present in all these selected plants. Saponins are absent in *Cinnamomum cassia*. Results of other phytochemical constituents are shown in **Table-3**. Quantitative evaluation of antibacterial activity (MIC) was carried out by microdilution method for methanolic and ethanolic extracts as these extracts have expressed strong antibacterial activity. Table-4 shows the MIC of selected plant extracts: *Cinnamomum cassia, Syzygium aromaticum,* and *Azadirachta indica* on five bacterial UTI isolates. A wide range of MIC values were recorded depending on the microbial strain.

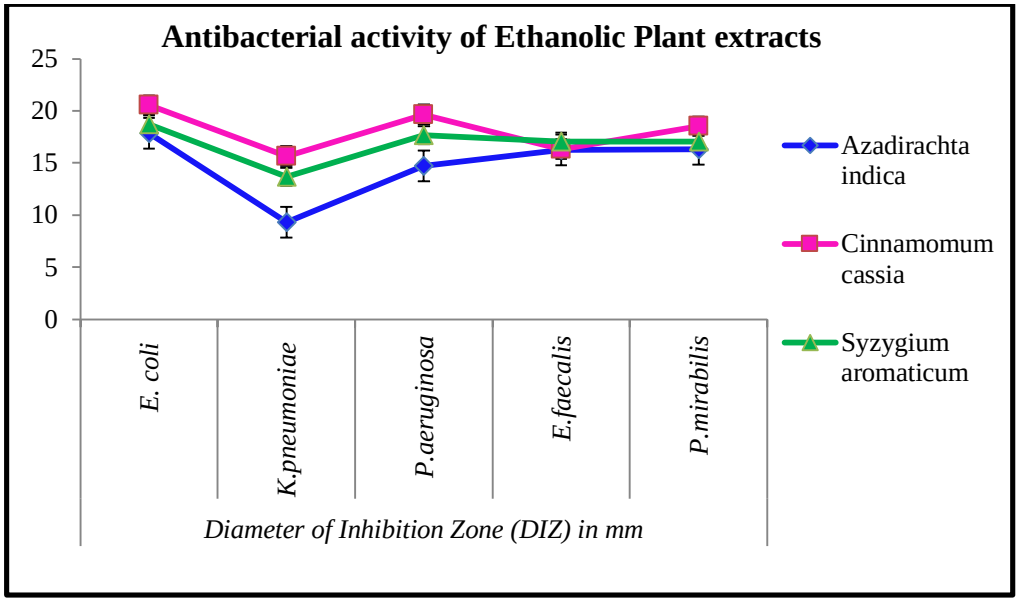

Figure 4: Antibacterial Activity of Ethanolic plant extracts on UTI Bacterial isolates

Table3: Phytochemical Analysis of Selected Plants

PLANT	TAN	ALK	FLAV	SAP	GLY	STER	PHEN
Azadirachta indica (leaf)	+	+	+	+	+	+	+
Cinnamomum cassia (bark)	+	+	+	-	+	+	+
Syzygium aromaticum (flower)	+	+	+	+	+	+	+

TAN- Tannins; ALK-Alkaloids; FLAV- Flavonoids; SAP-Saponins; GLY-Glycosides; STER-Steroids; PHEN-Phenolics.

Table4: MIC of Methanolic and Ethanolic plant extracts (mg/ml) on UTI Bacterial Isolates

Solvent	**Plant**	***E. coli***	***K. pneumoniae***	***P. aeruginosa***	***E. faecalis***	***P. mirabilis***
Methanolic	*Azadirachta indica* (leaf)	25	100	25	50	50
	Cinnamomum cassia (bark)	6.25	25	6.25	12.5	25
	Syzygium aromaticum (flower)	12.5	25	12.5	25	25
Ethanolic	*Azadirachtaindica* (leaf)	12.5	50	12.5	6.25	12.5
	Cinnamomum cassia (bark)	3.12	12.5	6.25	6.25	6.25
	Syzygium aromaticum (flower)	6.25	25	6.25	12.5	6.25

DISCUSSION

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. Urinary tract infection caused by bacteria is one of the serious issues which demand an urgent medical attention in community[36]. In the management of UTI patients the most effective approach is the identification of pathogens and selection of effective antimicrobial agent against them[37]. The traditional method for the diagnosis of UTI is plate count method in which $>10^5$ bacteria/mL of urine indicates bacteriuria[38, 39]. In the present study similar screening of UTI in urine samples was carried out for bacterial count by a quick and reliable method of urine dip slide (Thermo Fisher Scientific) which not only gives good results but also cut down the time consumed for plate count. The most foreseeable primary etiological agent involved in UTI is *E.coli*[40]. Similar results were obtained in our study in which *E. coli* was the most common bacteria isolated from positive urine sample(41.37%); this is in agreement with previous reports[41, 42].The second most common bacteria isolated in our study was *K. penumoniae* (25.86%) which is line with other reports [43]. The other three bacterial species- *P. aeruginosa*, *E. feaclis* and *P. mirabilis* were isolated with an isolation percentage of 5-20%.

Increasing resistance against antimicrobial agents is a worldwide problem. In this context one of the studies in India reported high prevalence rate of resistance towards the antibiotics which are commonly prescribed[44]. In support to this in our study we observed that bacterial isolates showed highest degree of resistance to gentamycin, nalidixic acid, trimethoprim-sulphamethaoxazole, clotrimazole and cefotaxime which are commonly prescribed for UTI treatment. Only three antibiotics (ampicillin, norfloxacin and tetracycline) out of 10 tested were found to be effective up to certain extent.

In the present study results obtained for antibacterial activity of plant extracts against clinical isolates suggest a class effect and indicate the superiority of these plant extracts compared to standard antibiotics tested. Antibacterial activity of aqueous, methanolic and ethanolic extracts of three plants: *Cinnamomum cassia* (bark), *Syzygium aromaticum* (flower) and ***Azadirachta indica*** **(leaf)** was tested on five bacterial isolates: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis* and *Proteus mirabilis* from patients suffering with UTI. Similar UTI pathogens have been reported by MN Ali and MM Khan[45]and **H.** Tabassum et al., [46]. The antibacterial strength was initially determined by the agar well diffusion method (as shown in Figures-2, 3 and 4) followed by quantitative evaluation by MIC method (as shown in Table-4).

Ethanolic extracts of all the three 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. aeruginosa* > *P. mirabilis* > *E. faecali s*> *K. pneumoniae.* Antibacterial activity of aqueous infusion, decoction and essential oil of *C. cassia* bark was investigated against 12 different genera of bacterial population isolated from oral cavity of 250 healthy individuals by NMA. Chaudhary and P. Tariq[47]. They have reported best effect of oil against all bacteria tested except *Salmonalle para typhi* B than aqueous decoction and Infusion. In another study on *Cinnamomum cassia* by Anjana Sharma *et al*.[48].highantibacterial activity was reported 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.

Next to *Cinnamomum cassia*, strong antibacterial effect was recorded for ethanolic extracts of *Syzygium aromaticum*- flower extracts. The order of inhibition followed a similarpattern to thatexhibited by *C. cassia* except for *E. faecalis* and *P. mirabilis* which were equally inhibited with a same DIZ of 17.06 ±0.11. *Syzygiumaromaticum*antibacterial activity of ethanolic and methanolic extracts with metal ion was tested on food borne pathogens by Pandey and Singh[49]. In their study compared to ethanolic extracts, methanolic extracts exhibited better antibacterial effect against Gram positive *S. aureus* and Gram negative *P. aeruginosa* and *E.coli*. They have reported positive results of metal ions (Zn^{++}, Cu^{++}, Pb^{++}, Mg^{++} and Fe^{++}) along with methanolic extracts against test organisms. The effectiveness of ethanolic extracts of *Syzygium aromaticum*-flower was also reported by **O.** Al-Jiffri *et al*.,[50]. The study was carried out on *E.* coli isolated from UTI samples and the antibacterial activity of cold water, boiling water and ethanolic extracts was investigated. High antibacterial activity was recorded for ethanolic extracts with a zone of 18 mm. These results are in line with results obtained in the present study and give a confirmation that ethanolic extracts have potential antibacterial effect.

The antibacterial activity of ethanolic extracts of *Azadirachta indica*– leaf expressed low antibacterial activity than the other two plant extracts. The inhibitory effect was observed to be highest 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. mirabilis > E. faecalis>P. aeruginosa> K. pneumoniae*. A study of bark, leaf, seed and fruit extracts of *Azadirachta indica* on clinical isolates from adult mouth was reported by Yerima et al., [51].The bark and leaf extracts showed antibacterial activity against all the tested bacteria (*P. aeruginosa, C. diptheriae* and *Bacillus spp.*). On contrary seed and fruit extracts showed activity only at higher concentrations. These results are in line with the results obtained in our study. Another report on effect of *Azadirachta indica* on clinical isolates of diabetic patients was given by Chaturvedi *et al*., [52]. The organisms tested were *S. aureus, Enterococcus, Escherichia, Pseudomonas* and *Klebsiella*. The results indicated high antibacterial activity of bark extracts than leaf against all bacteria tested.

The overall results obtained in the present research work gives a conclusive evidence of effectiveness with respect to antibacterial activity of the selected plant extracts on all five clinical bacterial isolates from urine samples with UTI. Among all the tested bacteria the lowest effect was recorded for *K. pneumonia* which gives a clue that capsule may be responsible for interfering factor towards the effectiveness of the components present in plant extracts. MIC by broth method showed good results compared to well diffusion method,this may due to poor diffusion of the biological components into the agar. The hydrocarbon components either remain on the surface of the medium or evaporate[53]. Broth method has advantage of use of lower volumesof the test extracts and growth medium for a large number of replicates[54]. The difference in the antibacterial activity of the same plant when extracted with different solvent has shown that phytochemicals which have a property of antibacterial activity are not soluble in a single solvent. This indicates that solvents of different polarity should be used as used in this study. The findings of the present study suggest that there is an urgent need for rigorous monitoring of antimicrobial substances used for treatment of urinary tract infections as 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[55]. In the present study ethanolic extracts of selected plants expressed strong and broad spectrum antibacterial activity to pathogenic UTI isolates. This may be due to presence of biologically active phytochemicals like flavonoids, alkaloids,

terpenoids, tannins etc., in these plants; and better extraction capacity of ethanol which might have yielded more number of active constituents responsible for antibacterial activity [56].

CONCLUSION

On account of results obtained we conclude that among the three selected plants used in this study *Cinnamomum cassia* and *Syzygium aromaticum* expressed broad spectrum antibacterial activity on all the five bacterial UTI isolates with highest activity recorded for ethanolic extracts. The antibacterial resistance shown by these clinical isolates towards standard antibiotics commonly used for UTI treatment suggests that, most of the antibiotics were ineffective in inhibiting the growth of these bacterial isolates. Importantly the results demonstrate that these plants contain biologically active substances which qualify them for medicinal use.

ACKNOWLEDGEMENTS

The authors are thankful to Principal and Management Committee, Mumtaz Degree & P.G. College, India for providing the facilities to carry out research work.

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On line publication Date: 6.5.2017