



## A STUDY OF UROPATHOGENS AND DETERMINATION OF IN VITRO ANTIMICROBIAL ACTION OF OCIMUM SANCTUM LINN. (TULSI) ESSENTIAL OIL ON THE ISOLATES FROM A TERTIARY CARE HOSPITAL.

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**ABSTRACT** **Purpose:** Urinary tract infection has always been a challenge in medical practice. The etiology and antimicrobial susceptibility pattern of uropathogens constantly change and thus periodic screening is essential. This study was conducted to analyse the profile and antibiogram of urinary tract pathogens in a tertiary care hospital, coastal, Karnataka, South India and to investigate the action of Tulsi (*Ocimum sanctum* Linn.) essential oil on the isolates. **Methods:** During the study period of one year, 288 clean catch midstream urine samples were cultured by semi- quantitative method and the colonies were identified using standard techniques. Antibiotic sensitivity testing was performed by Kirby-Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines. In vitro antimicrobial potential of the essential oil of Tulsi against the pathogens was determined by disk diffusion technique. **Results:** *Escherichia coli* was the predominant organism isolated from the samples which showed a prevalence of 35.85% followed by *Klebsiella* spp., 24.53%. Majority of the *Escherichia coli* isolates were resistant to Norfloxacin and Ampicillin. Most of the uropathogens in our study exhibited sensitivity to Tulsi essential oil. **Conclusions:** Analysis of the spectrum of uropathogens and their antibiotic sensitivity pattern in a particular locality is utmost necessary to decide on the empirical treatment of UTI. The phytochemicals present in plants possess antimicrobial properties which could be explored and utilized as they are cost effective and have lesser side effects.

**KEYWORDS :** Urinary tract infection, essential oil, disk diffusion technique

### INTRODUCTION

Urinary tract infection (UTI) is one of the frequently encountered infections in our clinical setting affecting all age groups. It affects millions of people worldwide annually which also imposes a huge financial burden on global economy.<sup>1</sup> UTIs account for about 40% of hospital acquired infections.<sup>2</sup>

Anatomically, infections of the urinary tract can be classified as upper UTI which involves the kidney or ureter and lower UTI which involves infection from the urinary bladder downwards.<sup>3</sup> UTIs are usually manifested as severe back pain, burning sensation while urinating, cloudy bad smelling urine, fever or chill.<sup>4</sup> It can also affect kidneys and can cause pyelonephritis. Women are more prone to UTI than men because of the proximity of their urethra to the anus and vagina which could serve as source for pathogens.<sup>5</sup> Kidney stones, urethral strictures, enlarged prostate, sexual activity, mode of birth control, menopause, diabetes and catheter use are the common predisposing factors for UTI.<sup>5,6</sup>

Bacteria, fungi, viruses and protozoan parasites have been implicated in causing UTI. *Escherichia coli* (*E. coli*) is the predominant etiological agent accounting for majority of UTI cases among the bacterial agents followed by *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Enterococcus faecalis*, *Staphylococcus saprophyticus* and *Staphylococcus aureus* (*S. aureus*).<sup>7,8</sup>

Resistance to antimicrobial agents used in the treatment of UTIs has been noted over decades and is a matter of concern. A study from southwest Ethiopia revealed a higher resistance rate to the commonly prescribed antibiotics.<sup>9</sup> It was observed that 100% of *E. coli* and *Klebsiella pneumoniae* isolates were resistant to Amoxicillin and Ampicillin which is of utmost significance.<sup>9</sup>

Most often, empirical treatment is initiated before urine culture and sensitivity results are available.<sup>7</sup> The frequent use & misuse of antibiotics has led to increased antibiotic resistance among the uropathogens which in turn complicated the treatment by increasing the costs, morbidity and by introduction of broad spectrum antibiotics.<sup>7</sup> The emergence of drug resistant uropathogens has triggered the need

for alternative treatment options.<sup>10</sup>

Plants as source of medicine have been utilised since ages as they are considered to be abundant in secondary metabolites and oils which are of therapeutic importance.<sup>11</sup> Essential oils extracted from aromatic medicinal plants have been reported to exhibit excellent antimicrobial effects against bacteria, yeasts, filamentous fungi, and viruses.<sup>12</sup> Plants contain numerous biochemical compounds to which microbes cannot develop resistance simultaneously.<sup>10</sup>

Among the medicinal plants, *Ocimum tenuiflorum*, also known as *Ocimum sanctum*, Tulsi, or Holy Basil from the family Lamiaceae has been regarded as the "Queen of plants" and "the mother medicine of nature" as it is a rich source of biologically active compounds.<sup>11,13</sup>

It has been a part of traditional medicine in India over the years and almost every part of the plant such as flower, fruit, leaf, stem and root is used as an expectorant, analgesic, anticancer drug, anti-asthmatic, antiemetic, diaphoretic, anti-diabetic, hepatoprotective, hypotensive and hypolipidemic.<sup>11</sup> Dark or Shyama (Krishna) Tulsi and light or Rama Tulsi are the two varieties which are commonly found in India and among these the former possesses greater medicinal value.<sup>11</sup>

The present study was intended to detect the uropathogens prevalent in our hospital and to determine their antibiotic susceptibility pattern. As the antibiogram of uropathogens changes depending upon the geographical area and due to development of new resistance mechanisms, periodic monitoring of the antibiotic susceptibility pattern is essential. Utilization of plant metabolites as alternative medicine in the era of drug resistant pathogens is highly promising as they are cost effective, easily available with lesser side effects. A preliminary in vitro analysis of the action of essential oil of *Ocimum sanctum* Linn. (Tulsi) on our isolates was also undertaken during the study period.

### MATERIALS AND METHODS

#### Sample Size

Sample size n (288) was calculated considering P = 58.71 with 10% available error (L), 95% confidence interval, using the formula,<sup>14</sup>

$$n = \frac{Z^2 \cdot 1 - \frac{\alpha}{2} \cdot P(1-P)}{L^2}$$

The present cross-sectional study was conducted at a tertiary care teaching hospital, coastal Karnataka, South India for a period of one year from May 2020 to May 2021.

The study was approved by the institutional ethics committee (AJEC/REV/042/2020).

Clean catch mid-stream urine samples of patients (both inpatients and outpatients) with suspected UTI attending our hospital were collected in properly labelled wide mouthed sterile containers with aseptic precautions. Patients on antibiotic therapy were excluded from the study. The samples were sent immediately to the Microbiology laboratory for further investigations.

**Processing**

Each urine specimen was examined macroscopically for colour and turbidity. After centrifugation, urine sediment was examined microscopically for pus cells, red blood cells, epithelial cells, cast & crystals.

Thoroughly mixed uncentrifuged sample was inoculated by the semi-quantitative method using the calibrated loop technique (0.001 mL) onto MacConkey agar (Microexpress, Tulip Diagnostics, Goa) & Cysteine lactose electrolyte deficient (CLED) medium (HiMedia, Mumbai) and was incubated aerobically at 37°C for 24 hours and in negative cases for 48 hours. Significant bacteriuria was considered as the pure growth of an isolate with a count ≥10<sup>5</sup> colony forming units (CFU) per milliliter of urine.<sup>7</sup>

Colonies were identified using colony morphology, Gram stain & standard biochemical tests.<sup>15</sup> Antibiotic sensitivity testing was performed on Mueller Hinton Agar by Kirby-Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines using E. coli (ATCC 25922), S. aureus (ATCC 25923) and Pseudomonas aeruginosa (P. aeruginosa) (ATCC 27853) as control strains.<sup>16</sup>

For the identification of Candida spp., HiCrome Candida differential agar (HiMedia, Mumbai) was employed and Candida albicans (C. albicans) isolates were confirmed by Germ tube test.

**Antibiotic Discs Employed**

The following antibiotic discs (HiMedia, Mumbai) were employed in this study. Ampicillin (10mcg), Amoxyclav (30mcg), Amikacin (30mcg), Gentamicin (10mcg), Cefoperazone- sulbactam (75mcg), Imipenem (10mcg), Piperacillin- tazobactam (100mcg), Cotrimoxazole (25mcg), Nitrofurantoin (300mcg), Norfloxacin (10mcg), Linezolid (30mcg), Vancomycin (30mcg), Clindamycin (2mcg), Meropenem (10mcg), Aztreonam (30mcg), Levofloxacin (5mcg), Cefepime (30mcg), Ceftazidime (10mcg).

**Essential oil**

Essential oil (100% pure steam distilled) of Holy Basil procured from Veltek India was used in the study.

**In Vitro Antimicrobial Action Of Tulsi Essential Oil**

Stock cultures of the uropathogens obtained were maintained on nutrient agar slopes and stored at 4°C for determination of in vitro antibacterial action of the Tulsi essential oil. In vitro antibacterial potential of the essential oil of Tulsi against the pathogens was determined by disk diffusion technique.<sup>17</sup>

A 4-6 hour old culture of the isolates in Mueller Hinton broth, the turbidity of which was adjusted to McFarland 0.5 standard (1.5x10<sup>8</sup> colony forming units/ml) was swabbed onto Mueller Hinton agar plates. One ml from the undiluted essential oil of Tulsi were added to 100 sterile discs (Whatman No.1 filter paper) of 6mm diameter each in a sterile petri plate. These discs were then placed on the inoculated Mueller Hinton agar plates.<sup>18</sup>

The zone of inhibition in millimetres was measured after incubation at 37°C for 24 hours.

E. coli (ATCC 25922), S. aureus (ATCC 25923) and P. aeruginosa (ATCC 27853) were also employed in parallel. Dimethyl sulfoxide (DMSO) incorporated disc was included as a negative control. The

results were graded depending on the diameter of the zones of inhibition.

9 mm – 12 mm → 1+, 13 mm – 16 mm → 2+, 17 mm – 20 mm → 3+, >20 mm → 4+.<sup>18,19</sup>

**Statistical Analysis**

Data were analysed by frequency, percentage and Chi-square test.

**RESULTS**

A total of 288 urine samples were processed during a period of one year from May 2020 to May 2021. Out of this 53 (18.4%) samples were culture positive & 235 (81.6%) samples did not show any growth. Among 288 patients, 167 (57.99%) were females & 121 (42.01%) were males. Out of this, 31 (18.56%) samples showed growth in culture among females & 22 (18.2%) were culture positive among males and no statistically significant difference was observed among the gender (P=0.934, NS) (Table 1).

**Table 1: Culture Positivity Based On Gender**

Sex	No. of patients (n=288)	No. of culture positives (53)
Females	167	31 (18.56%)
Males	121	22 (18.2%)
Statistical analysis (by Chi-square test)	P=0.934, NS	

NS=Not significant

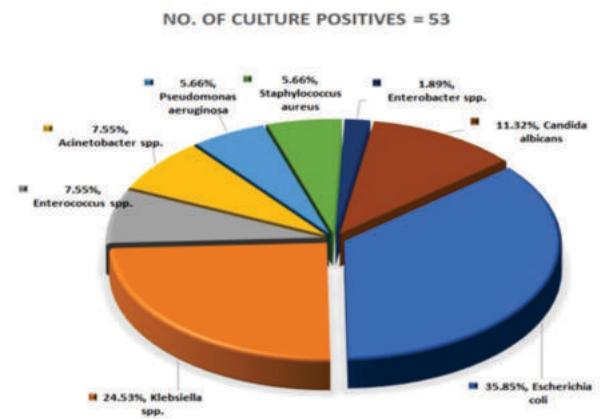
On the basis of age wise distribution of our patients, about 10 (3.5%) were paediatric patients (between 0 to 14 years), 147 (51.04%) patients were between 15 and 47 years of age, 78 (27.08%) patients belonged to 48 to 63 years of age and 53 (18.4%) patients were equal to or above 64 years of age. From the total 53 culture positives, the age groups 15 to 47, 48 to 63 and ≥64 displayed culture positivity of 22 (41.97%), 17 (21.79%) and 14 (26.42%) respectively. There were no culture positives in the age group 0-14 years. The difference in culture positivity among the different age groups were not statistically significant (P=0.099, NS) (Table 2).

**Table 2: Culture Positivity Based On Age Groups**

Age groups	No. of patients (n=288)	No. of culture positives (53)
0-14 years	10	0 (0%)
15-47 years	147	22 (14.97%),
48-63 years	78	17 (21.79%)
≥64 years	53	14 (26.42%)
Statistical analysis (by Chi-square test)	P=0.099, NS	

NS: Not significant

E. coli was the predominant organism isolated from our samples which showed a prevalence of 19 (35.85%) followed by Klebsiella spp. 13 (24.53%), Enterococcus spp. 4 (7.55%), Acinetobacter spp. 4 (7.55%), P. aeruginosa 3 (5.66%), S. aureus 3 (5.66%), and Enterobacter spp. 1 (1.89%). C. albicans isolated in our study was 6 (11.32%) (Figure 1).



**Fig 1: Organisms Isolated From The Urine Samples**

Out of 19 isolates of *E. coli*, 17 (89.47%) were found to be sensitive to Nitrofurantoin. Fifteen (78.95%) isolates each were sensitive to both Amikacin and cefoperazone-sulbactam. Seventeen (89.47%) isolates were resistant to Ampicillin and 15 (78.95%) isolates were resistant to Norfloxacin. Among 13 isolates of *Klebsiella* spp., 11 (84.62%) and 10 (76.92%) were sensitive to Nitrofurantoin and Amikacin respectively. Twelve (92.31%) isolates were resistant to Ampicillin (Table 3).

**Table 3: Antibiogram Of The Predominant Uropathogens (gram Negative Bacteria) Isolated**

Antibiotics	Escherichia coli (n=19)		Klebsiella spp. (n=13)	
	Sensitivity (No. & %)	Resistance (No. & %)	Sensitivity (No. & %)	Resistance (No. & %)
Ampicillin	2 (10.53%)	17 (89.47%)	1 (7.69%)	12 (92.31%)
Amoxycylav	11 (57.89%)	8 (42.11%)	7 (53.85%)	6 (46.15%)
Amikacin	15 (78.95%)	4 (21.05%)	10 (76.92%)	3 (23.08%)
Gentamicin	13 (68.42%)	6 (31.58%)	7 (53.85%)	6 (46.15%)
Cefoperazone-sulbactam	15 (78.95%)	4 (21.05%)	7 (53.85%)	6 (46.15%)
Imipenem	13 (68.42%)	6 (31.58%)	8 (61.54%)	5 (38.46%)
Piperacillin-tazobactam	12 (63.16%)	7 (36.84%)	7 (53.85%)	6 (46.15%)
Co-trimoxazole	11 (57.89%)	8 (42.11%)	7 (53.85%)	6 (46.15%)
Nitrofurantoin	17 (89.47%)	2 (10.53%)	11 (84.62%)	2 (15.38%)
Norfloxacin	4 (21.05%)	15 (78.95%)	8 (61.54%)	5 (38.46%)

All the three (100%) isolates of *S. aureus* showed sensitivity to Linezolid. Three (75%) isolates of *Enterococcus* spp. exhibited sensitivity to both Linezolid and Vancomycin. Among the four isolates of *Enterococcus* spp. 3 (75%) isolates each were resistant to Erythromycin and Tetracycline (Table 4).

**Table 4: Antibiogram Of The Predominant Uropathogens (gram Positive Bacteria) Isolated**

Antibiotics	Staphylococcus aureus (n=3)		Enterococcus spp. (n=4)	
	Sensitivity (No. & %)	Resistance (No. & %)	Sensitivity (No. & %)	Resistance (No. & %)
Linezolid	3 (100%)	0 (0%)	3 (75%)	1 (25%)
Vancomycin	2 (66.67%)	1 (33.33%)	3 (75%)	1 (25%)
Clindamycin	1 (33.33%)	2 (66.67%)	2 (50%)	2 (50%)
Erythromycin	2 (66.67%)	1 (33.33%)	1 (25%)	3 (75%)
Tetracycline	2 (66.67%)	1 (33.33%)	1 (25%)	3 (75%)
Ampicillin	1 (33.33%)	2 (66.67%)	2 (50%)	2 (50%)
Ciprofloxacin	1 (33.33%)	2 (66.67%)	2 (50%)	2 (50%)

All the three isolates (100%) of *P. aeruginosa* were sensitive to imipenem and all the isolates (100%) displayed resistance to piperacillin-tazobactam and meropenem. Two isolates (66.67%) were also resistant to Aztreonam. All the four isolates (100%) of *Acinetobacter* spp. showed sensitivity to Amikacin. Three isolates (75%) of *Acinetobacter* spp. were sensitive to levofloxacin. Two (50%) isolates of *Acinetobacter* spp. were sensitive to Gentamicin, meropenem and cefepime. Only one *Enterobacter* spp. was isolated, which showed sensitivity to Co-trimoxazole, Piperacillin-tazobactam, Amikacin and Ceftazidime and was resistant towards Amoxycylav & Gentamicin.

Out of 19 isolates of *E. coli*, all (100%) were sensitive to Tulsi essential oil by disk diffusion. Seven isolates (36.84%) showed sensitivity in 1+ category and twelve isolates (63.16%) exhibited sensitivity in 2+ category. Among 13 isolates of *Klebsiella* spp., all (100%) were sensitive to the essential oil of Tulsi by disk diffusion. Five (38.46%) isolates belonged to 1+ range and the remaining 8 (61.54%) belonged to 2+ range. Out of the 4 isolates of *Enterococcus* spp., 2 (50%) were sensitive & 2 (50%) were resistant to Tulsi essential oil. The two sensitive isolates showed 1+ sensitivity. Out of the 4 *Acinetobacter* spp. isolated all (100%) exhibited sensitivity towards the essential oil. 3 isolates (75%) were sensitive in the 1+ range and 1 isolate (25%) was sensitive in the 2+ range. Out of the 3 *P. aeruginosa* isolates, only one (33.33%) was sensitive and two isolates (66.67%) were resistant to the oil. One sensitive isolate exhibited zone of inhibition in the 1+ range. All the 3 (100%) *S. aureus* isolates exhibited sensitivity to Tulsi oil and all the three (100%) belonged to 1+ category. One *Enterobacter* spp. isolated was sensitive to Tulsi oil in the 1+ range. All the six (100%) *C. albicans* isolates were sensitive to the essential oil in the 3+ range

(Table 5).

**Table 5: In Vitro Antimicrobial Action Of Ocimum Sanctum Linn. (tulsi) Essential Oil Against The Isolates**

No. of isolates tested	Sensitive isolates		Total no. of sensitive isolates	Resistant isolates
Escherichia coli (19)	1+	7 (36.84%)	19 (100%)	Nil
	2+	12 (63.16%)		
	3+	0 (0%)		
	4+	0 (0%)		
Klebsiella spp. (13)	1+	5 (38.46%)	13 (100%)	Nil
	2+	8 (61.54%)		
	3+	0 (0%)		
	4+	0 (0%)		
Enterococcus spp. (4)	1+	2 (100%)	2 (50%)	2 (50%)
	2+	0 (0%)		
	3+	0 (0%)		
	4+	0 (0%)		
Acinetobacter spp. (4)	1+	3 (75%)	4 (100%)	Nil
	2+	1 (25%)		
	3+	0 (0%)		
	4+	0 (0%)		
Pseudomonas aeruginosa (3)	1+	1 (100%)	1 (33.33%)	2 (66.67%)
	2+	0 (0%)		
	3+	0 (0%)		
	4+	0 (0%)		
Staphylococcus aureus (3)	1+	3 (100%)	3 (100%)	Nil
	2+	0 (0%)		
	3+	0 (0%)		
	4+	0 (0%)		
Enterobacter spp. (1)	1+	1 (100%)	1 (100%)	NIL
	2+	0 (0%)		
	3+	0 (0%)		
	4+	0 (0%)		
Candida albicans (6)	1+	0 (0%)	6 (100%)	Nil
	2+	0 (0%)		
	3+	6 (100%)		
	4+	0 (0%)		

Key : 9-12 mm = 1+, 13-16 mm = 2+, 17-20mm = 3+, >20 mm = 4+

## DISCUSSION

UTI is one of the most common infections dealt with in routine medical practice which infects millions worldwide. The present study was conducted to identify the urinary pathogens from the samples submitted to our Microbiology laboratory and to analyse their antibiogram. A preliminary attempt was also made to examine the in vitro antimicrobial activity of Tulsi on these isolates which could aid in development of alternative therapy for drug resistant uropathogens.

During the study period, the culture positivity obtained was 18.4% whereas Mohapatra et al reported a culture positivity rate of 10.1%.<sup>8</sup> Another study from Uganda reported significant bacterial growth in 22.33% of samples whereas a culture positivity rate of 41.8% was obtained in an Indian study.<sup>7,10</sup> Higher prevalence rate of 53.82% was displayed by another study from India.<sup>1</sup>

In our study, among females, 18.56% samples showed growth in culture and 18.2% were culture positive among males. The difference in culture positivity between males and females was not statistically significant in our study (P=0.934, NS). This completely correlated to a study by Tibyangye and colleagues where no much difference was obtained between the culture positivity among males (21.15%) and females (22.96%).<sup>10</sup> Our finding was not in agreement with Prakash and Saxena who reported significantly higher prevalence rate of UTI in female patients (73.57%) when compared to males (35.14%).<sup>1</sup>

According to the age groups employed in our study, 15 to 47, 48 to 63 and ≥64 displayed culture positivity of 22 (14.97%), 17 (21.79%) and 14 (26.42%) respectively. There were no culture positives in the age group 0-14 years. In this study, the difference in culture positivity among the different age groups was not statistically significant (P=0.099, NS). There was no statistically significant difference in culture positivity among different age groups in an Ethiopian study whereas a study from Uganda observed a high prevalence of UTI

(46.27%) in the age group 18-28 years.<sup>9, 10</sup> A south Indian study demonstrated highest prevalence of 42.1% in the age group greater than 60 years.<sup>20</sup>

*E. coli* was the predominant organism isolated from our samples which showed a prevalence of 35.85% followed by *Klebsiella* spp. (24.53%). This is in complete agreement with a study conducted on multidrug resistant uropathogens where the primary isolates were found to be *E. coli* (55.61%) and *Klebsiella pneumoniae* (16.7%).<sup>20</sup> *E. coli* (42.58%) and *Klebsiella pneumoniae* (18.71%) were the most prevalent bacteria according to another study conducted in an Indian city.<sup>1</sup> *C. albicans* isolated in our study was 11.32% which correlated with the isolation rate from another study.<sup>7</sup>

In the present study, 89.47% of *E. coli* were found to be sensitive to nitrofurantoin and 78.95% isolates were resistant to norfloxacin which is in complete agreement with the study conducted by Singhal and colleagues which revealed high susceptibility of *E. coli* to nitrofurantoin (90.6%) and low susceptibility to norfloxacin (19.9%).<sup>7</sup> But this is in disagreement with Arjunan and colleagues who reported lowest resistance to norfloxacin (5.56%). 89.47% of our *E. coli* isolates were resistant to ampicillin which is in accordance with their study which showed ampicillin resistance in 93.1% of the isolates.<sup>21</sup> The sensitivity exhibited towards both Amikacin (78.95%) and cefoperazone- sulbactam (78.95%) by the *E. coli* isolates in our study was similar to another Indian study.<sup>7</sup>

The second most prevalent isolate was *Klebsiella* spp. and 92.31% of the isolates were resistant to ampicillin which is in accordance with the results of Beyene and Tsegaye which indicated hundred percent resistance to ampicillin in their study.<sup>9</sup> Our study revealed that 76.92% of our isolates were sensitive to amikacin and a study from Pakistan reported that the lowest resistance observed among *Klebsiella* spp. was against amikacin (37%).<sup>22</sup>

All the three isolates (100%) of *P. aeruginosa* were sensitive to imipenem in our study and this is in accordance with a study from Pakistan which reported low resistance (33.3%) to imipenem by the bacteria.<sup>22</sup> This is also consistent with the findings of a north Indian study according to which imipenem inhibited 90.5% of *P. aeruginosa*.<sup>23</sup> In the present study all the isolates (100%) of *P. aeruginosa* displayed resistance to piperacillin –tazobactam. This is in contrast to a few authors who found that it inhibited 71.4% of *P. aeruginosa*.<sup>23</sup>

A few authors demonstrated 100% sensitivity of *Staphylococcus* spp. to vancomycin whereas only 66.67% of our isolates were inhibited by the drug.<sup>7, 23</sup> In our study vancomycin sensitivity was shown by 75% of *Enterococcus* spp.

which is similar to 76.9% sensitivity by the bacteria as demonstrated by Singhal and colleagues [7]. Linezolid inhibited 75% of our *Enterococcus* spp. which is in compliance with a study which indicated 88.9% susceptibility to the antibiotic.<sup>23</sup>

Emerging mechanisms of resistance among the pathogens pose a great threat for treatment of most of the infections. Researchers are in constant search for alternative options which are economical with lesser side effects to combat this problem. Plant extracts and essential oils have been essential elements of traditional medicine in India for treatment of variety of infections.

Holy Basil also known as Tulsi (*Ocimum sanctum*) is an aromatic herb with excellent antimicrobial activities against many pathogens and is increasingly used for wound healing and preservation of food stuff. Tulsi contains natural compounds like terpenoids, alkaloids, glycosides, tannins and flavonoids.<sup>24</sup> Tulsi has been used in different forms such as extract and essential oil in medicines in India from time immemorial.<sup>25</sup>

We observed good antimicrobial activity of Tulsi essential oil in vitro against the urinary isolates tested in our study. It was demonstrated that different concentrations of essential oil of Tulsi employed in a study completely inhibited the growth of *S. aureus* and *E. coli* but was less effective against *P. aeruginosa* which is in agreement with our results.<sup>13</sup> Malik & Singh also observed similar result in their study.<sup>26</sup> In an in vitro study conducted on uropathogens, it was proved that *Ocimum suave* essential oil exhibited good activity against *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, *Enterococcus faecalis*, *Morganella morganii*, *Citrobacter* spp., *Enterobacter* spp. and *P. aeruginosa* but showed no

activity against *Acinetobacter* spp.<sup>10</sup> Another study reported excellent antibacterial activity of methanolic extract of *Ocimum sanctum* against *Klebsiella* spp.<sup>27</sup> Mittal and colleagues studied the antimicrobial activity of *Ocimum sanctum* leaf extracts and found that ethanol and chloroform extracts exhibited excellent antibacterial activity against *S. aureus* and *Klebsiella pneumoniae*.<sup>28</sup>

We demonstrated excellent antimicrobial activity of Tulsi essential oil against *C. albicans* which is well supported by a study from New Delhi, India according to which this essential oil was the most effective among other essential oils against both *C. albicans* and *Candida tropicalis*.<sup>29</sup>

## CONCLUSION

The present study was intended to analyse the routine pathogens isolated from urine samples submitted to our Microbiology laboratory and to determine their antimicrobial susceptibility pattern. Due to diverse drug resistance mechanisms among the urinary pathogens, it becomes imperative to constantly screen for the susceptibility of the organisms to drugs commonly prescribed for empirical treatment of UTI. *E. coli* was the predominant pathogen isolated in our study and majority of the isolates were resistant to Norfloxacin. *E. coli* as well as *Klebsiella* spp., which was the next most prevalent uropathogen exhibited resistance to Ampicillin. The reduced susceptibility to these drugs indicate their indiscriminate usage and new resistance mechanisms among the microbes.

As plant phytochemicals could serve as alternative medicine for treatment of various illnesses, we attempted a preliminary in vitro analysis using the essential oil of Tulsi (*Ocimum santum* Linn.) on these uropathogens. Our results demonstrated that Tulsi oil exhibited good inhibitory activity on majority of the isolates which shows that it has the potential to serve as curative against UTI. In vitro studies with more number of isolates and also in vivo analysis have to be performed before implementing it in medical practice.

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