Antimicrobial Activity of Traditional Medicinal Plants Available at Banepa and Bhaktapur against Uropathogens

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ABSTRACT

Objectives: The study was aimed to determine the antimicrobial activity of traditional medicinal plants against the uropathogens.

Methods: Overall, 360 urine samples were collected from both outpatient and inpatient for culture and antimicrobial susceptibility testing. All the isolates were processed and identified following standard microbiological procedure and subjected to antibiotic susceptibility testing at Microbiology laboratory of Shree Birendra Hospital following CLSI guidelines. All the three plant extracts were processed by agar well diffusion method and Tube dilution method for antimicrobial activity against *Escherichia coli, Klebsiella. pneumoniae, Pseudomonas aeruginosa and Enterobacter* spp. at Microbiology laboratory of Sainik Awasiya Mahavidhyalaya following standard laboratory techniques.

Results: Crude extract of plants viz. *Centella asiatica, Cuscuta reflexa and Mentha spicata* showed good antimicrobial properties against all clinical isolates. Among all plants, ethanolic extract of *C. asiatica* was found to be most effective against *E. coli* with zone of inhibition 16 mm and minimum inhibitory concentration (MIC) value 5 mg/ml. Acetone extract of *C. reflexa* showed good antimicrobial activity against *K. pneumoniae* with zone of inhibition 14 mm and MIC value 10 mg/ml.

Conclusion: Our research revealed that the crude plant extracts, particularly the acetone and ethanol extracts, had a considerable amount of efficacy against uropathogens. Based on the study results, these traditionally used medicinal plants can overcome the problems of infections caused by multidrug resistant bacteria.

Keywords: Urinary tract infection, antimicrobial activity, Medicinal plant, uropathogens, multidrug-resistant

INTRODUCTION

Urinary Tract Infection refers to the occurrence of bacterial pathogens in the urine or tissue of the typically sterile genitourinary system (Nicolle, 2012). It is the most prevalent bacterial infection that affects millions of people worldwide each year (Foxman and Brown, 2003) in all age groups (Prakash et al. 2011) across their entire life and resulting in heightened rates of illness and deaths (Basnet et al. 2009). Each year, about 150 million individuals worldwide receive a diagnosis of UTIs, with the global economy surpassing a colossal sum of 6 billion dollars in US (Gonzalez and Schaeffer, 1999). UTIs include a variety of diseases caused by the invasion of microorganisms into the genitourinary tract, ranging from renal cortex infections of the kidney to urethral meatus infections (Mahon et al. 2007). The two most significant signs of urinary tract infections are pyuria and bacteriuria (Douri, 2008). Bacteriuria is

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characterized by the presence of more than 10^5 colonies of a single pathogen per milliliter of urine (Çelen et al. 2011) and the term "pyuria" refers to the presence of white blood cells (WBCs) in a person's urine (Adegoke et al. 2012). UTI is indeed a prevalent illness among the people of Nepal too and is also one of the prevailing nosocomial (hospital-acquired) infections (Kattel et al. 2008). As per the annual report released by the Department of Health Services in Nepal for the year 2059/60, the morbidity rate of UTIs was documented as 1,250,584. UTIs are more common in women compared to men. It is estimated that approximately one out of three women will have encountered a minimum one episode of UTI necessitating antimicrobial treatment by the age of 24 years. According to report nearly half of all women will undergo at least one UTI at some point in their lives (Foxman, 2002), this results from anatomical predisposition or urothelial mucosal adhesion to the mucopolysaccharide lining (Öztürk and Murt, 2020).

UTIs can be caused by various gram-negative bacteria that enter the urinary tract and establish a significant presence of bacteria in the urine. Among the different bacterial pathogens, *Escherichia coli* is the predominant one followed by *Staphylococcus saprophyticus* which accounts for approximately 10% to 30% of infections in young and adult females. In addition, other members of the Enterobacteriaceae family, including *Klebsiella* spp, *Proteus* spp, *Enterobacter*, and *Pseudomonas* spp. are commonly associated with UTIs in individuals with compromised immune systems or underlying medical conditions that increase susceptibility to infections (Svanborg and Godaly, 1997).

The widespread use of effective antibiotics is being greatly restricted by the global expansion of multi-drug resistant microorganisms (Alekshun and Levy, 2007). The World Health Organization (WHO) has called antibiotics resistance an emerging disease (Omigie et al. 2009). The origin of antibiotics resistance among uropathogens is a significant health problem especially in developing nations where people are at a much higher level of poverty and unsanitary habits. Nowadays it is difficult to treat UTI by using chemotherapy (Soulsby, 2005). Over the past century, antibiotics have significantly increased life expectancy and saved the lives of millions of people. Nevertheless, the rise of multi-drug resistant (MDR) bacteria is endangering the therapeutic effectiveness of many of the current antibiotics (Bandow et al. 2003). In addition, the infections with MDR strains are linked to longer hospitalizations, higher healthcare costs, and higher rates of morbidity and death.

Throughout human history, herbal treatments have been used to treat a wide range of infectious diseases. Even in many underdeveloped nations today, plant materials are still widely used as therapeutic medicines in primary healthcare (Zakaria 1999). Phytochemicals found in medicinal plants are a valuable resource for traditional medicinal treatment of a wide range of ailments (Shrestha et al. 2015). Medicinal plants are regarded as abundant sources of components that can be used in the pharmaceutical, non-pharmacopoeia, or synthetic drug industry. Nepal has a wide range of physiographic and climate variables along its height gradient. Based on information from multiple sources, the number of medicinal and aromatic plant species in Nepal is believed to be between 700 and 1,600. Manandhar and Manandhar, 2002 has reported ethno-botanical information of 1,500 plant species, majority of them have medicinal value. A wide range of medicinal plants parts is used to extract as raw drugs and they possess varied medicinal properties (Hashim EL-Kamali & Yagoub EL-amir, 2010). Hence, in our study, we introduced 3 traditionally used medicinal plants and extracted the active components to determine the antimicrobial activity against bacterial pathogens including uropathogens and pus isolates. The extracts of those plants viz: *Centella asiatica, Cuscuta reflexa,* and *Mentha spicata* having higher antimicrobial activity could be used as an alternative of antibiotics to treat MDR associated infections

MATERIALS AND METHODS

Study design, study site and study population: A cross-sectional study was conducted among the patients visiting Shree Birendra Hospital, Chhauni, Nepal. The clinical samples from patients were processed at the Microbiology laboratory of the Hospital and the remaining work on antimicrobial activity of medicinal plants against the uropathogens was carried out at Microbiology laboratory of Sainik Awasiya Mahavidhyalaya, Bhaktapur.

Sample collection, transport and processing: A total of 360 urine samples were collected in sterile container and transported to the laboratory. The medicinal plants were collected from different sites of Banepa and Bhaktapur and were transported to the Microbiology

Laboratory, Sainik Awasiya Mahavidhyalaya for further processing.

Ethical consideration: Ethical approval was obtained from the Institutional Review Committee (IRC), Institute of Science and Technology, Tribhuvan University (Reg no.: IRCIOST-23-0058). Permission for data and sample collection were taken from the authority of SBH after submitting a written request letter from the college.

Laboratory procedure of clinical samples

Isolation and identification of bacteria from samples: A loopful of urine was streaked on the CLED agar and incubated at 37°C for 24 hours to isolate the UTI-causing bacterium (Inabo and Obanibi, 2006). The suspected colonies were sub-cultured on Nutrients Agar and incubated for 24 hours at 37°C. The potential bacterial pathogens were identified by using standard microbiological procedure including morphological, cultural and biochemical characteristics (Cheesebrough 2006). Other bacterial pathogens including *P. aeruginosa* and *Enterobacter* spp. were obtained from pus isolates.

Antibiotic susceptibility testing: The identified bacterial isolates were subjected to antibiotic susceptibility testing by modified Kirby Bauer disc diffusion method on Mueller Hinton Agar (MHA) plates following CLSI guidelines (CLSI 2019). Similarly, the isolates that were resistant to 3 or more different classes of antibiotics were considered multidrug-resistant strains ((Magiorakos et al. 2012)

Laboratory procedure of medicinal plants

Collection and Identification of Medicinal Plants: The plant samples were collected from different sites of Banepa and Bhaktapur. The taxonomic identification of the collected medicinal plants was confirmed at Central Department of Botany, Tribhuvan University.

Transportation and preliminary processing of the plants: Three different plants *Centella asiatica (Ghodchhapre), Cuscuta reflexa (Aakashbeli), and Mentha spicata (pudina),* their various parts like whole plant and leaves individually were gathered in view of ethnomedical significance and data given by nearby medication men of Nepal. The plant samples were collected were processed at Microbiology Laboratory, Sainik Awasiya Mahavidhyalaya Bhaktapur, Nepal. By utilizing a mortar and pestle, the medicinal herbs were individually air dried and then finely powdered. This powder was kept in store in cold temperature with proper labeling.

Extraction of antibacterial compounds in solvent: The extraction was done by solid-liquid extraction technique by using soxhlet extractor. Ten-gram dried powder of each medicinal plant were weighed and placed in an extraction chamber, which would be placed on top of a collecting flask and below a reflux condenser, in a cellulose thimble. A suitable solvent (aqueous water, acetone, and ethanol) was added to the flask, and the set up was heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it was siphoned into the flask beneath. Then, the obtained solution was mixed well and filters using Whatman's no.1 paper. Different plant compounds which were suspended in solvent were taken and evaporate it by resting extract for 3-4 days without cover. After 3-4days, the powder was obtained and dissolved in Dimethyl Sulfoxide (DMSO) which dissolves both polar and non-polar compounds. The concentration in mg/ml was made from it (Nair et al. 2005). The extracts were labeled and kept in the refrigerator at 4°C until used.

Antimicrobial activity of plant extracts: The agar well diffusion method was employed in sterile MHA plates to test the antimicrobial activity of plant extracts. The fresh test cultures were incubated at 37°C for a few hours to obtain turbidity equivalent to 0.5 McFarland's standard to produce a final concentration of 1.5×10^8 CFU/ml and swabbed onto the MHA plate. A sterile cork-borer (5 mm) was used to form wells in the inoculated media. The width of the inhibitory zone was measured in mm after each well had been filled with 40 µl of various plant extract concentrations and incubated at 37°C for 24 hours. Chloramphenicol (30 mcg) was used as positive control and DMSO as negative control.

Minimum Inhibition Concentration (MIC): *C. asiatica*, *C. reflexa*, and *M. spicata* extracts were made at different concentrations (20, 10, and 5 mg/mL), and serial dilutions of the extracts were prepared and placed in test tubes. Using non-toxic pipette tips, bacterial inoculation was inoculated into Mueller Hinton broth. Then 0.1 ml of different concentrations (20, 10 and 5 mg/ml) of each extract were added to each test tube and incubated overnight at 37°C. The minimum inhibitory concentration was considered to be the lowest concentration that inhibited microbial development.

Quality Control: Positive and negative controls were used in each test.

RESULTS

Growth pattern of bacteria in specimen: Out of 360 urine samples, significant growth was observed among 15(4.2%) samples while 33(9.2%) showed mixed growth and 312(86.7%) showed non-significant growth (Figure 1).

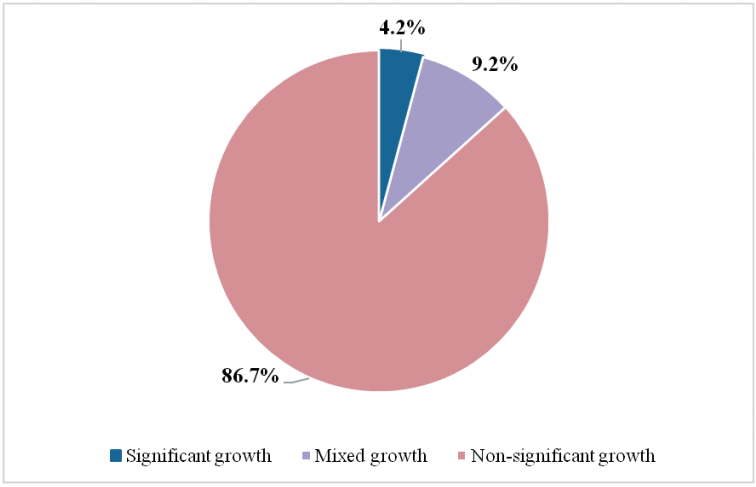

Figure 1: Growth pattern of bacteria in specimen

Gender and age wise distribution of UTI: The highest percent of growth was observed in the samples from females of age group NB-19 (13.0%). Similarly, the higher infections were observed in males of age group greater than 80 years (6.3%) (Table1).

Table 1: Gender and age-wise distribution of the infection

Age (years)	Female		Male		Total	p-value
	No.	%	No.	%		
NB-19	3	13.0	0	0	3(7.5)	
20-39	3	4.8	0	0	3(2.8)	
40-59	3	7.6	0	0	3(4.2)	0.2
60-79	2	3.9	2	3.3	4(3.5)	
>80	1	8.3	1	6.3	2(7.1)	
Total	**12**	**6.4**	**3**	**1.7**	**15(4.2)**	

Note: NB-Newborn

Ward- wise distribution of the infection: Out of total samples, majority of the patients requested for urine culture were outpatients which accounted for about 82.5% (297/360). The remaining 17.5% (63/360) were inpatients and the rate of UTI was higher among out-patients (4.4%) in comparisons to in-patients (3.2%) which is statistically not significant ($p > 0.05$) (Table 2).

Table 2: Ward-wise distribution of the infection

Inpatient/Outpatient	Total No.	Significant growth N (%)	P-value
Inpatient	63	2(3.2)	
Outpatient	297	13(4.4)	0.7
Total	**360**	**15(4.2)**	

Distribution of bacterial isolates: Out of 15 bacterial isolates, all the isolates were Gram negative organism (100%). Among total isolates of Gram-Negative bacteria, *E. coli* was found to be 11(73.3%) and *K. pneumoniae* 4 (26.7%) (Figure 3).

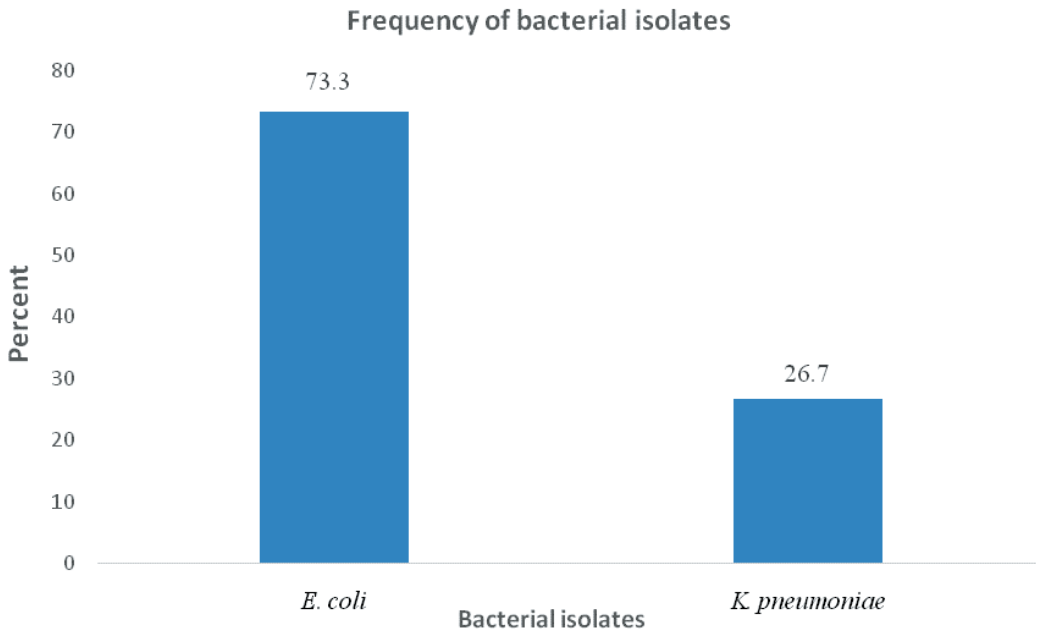

Figure 2: Frequency of bacterial isolates from UTIs

Antibiotic susceptibility pattern of *E. coli*: Out of 11 *E. coli,* chloramphenicol (100%) was found to be most effective drug followed by Tetracycline (72.7%) and Piperacillin/ tazobactam (57.1%). Similarly, Cefotaxime and Cefixime (9.1%) was found to be least sensitive (Table 3, Figure 3).

Table 3: Antibiotic Susceptibility pattern of *E. coli* (N=11)

Groups of Antibiotics	Antibiotics used	Susceptibility Pattern			
		Resistant		Susceptible	
		No. (%)	%	No.	%
Penicillin	Ampicillin	8	72.7	3	27.3
	Piperacillin	2	66.7	1	33.3
ß-lactamase Inhibitor	Amoxycillin/Clavulanic acid	9	81.8	2	18.2
	Piperacillin/Tazobactam	3	42.9	4	57.1
Cephalosporin	Cefotaxime	10	90.9	1	9.1
	Cefixime	10	90.9	1	9.1
	Ceftriaxone	9	81.8	2	18.2
	Cefepime	6	85.7	1	14.3
	Ceftazidime	6	85.7	1	14.3
Cephalosporin	Imipenem	4	57.1	3	42.9
	Meropenem	4	57.1	3	42.9
Nitrofurans	Nitrofurantoin	5	45.4	6	54.6
Aminoglycosides	Gentamycin	5	45.4	6	54.6
	Amikacin	8	72.7	3	27.3
Tetracycline	Tetracycline	3	27.3	8	72.7
	Doxycycline	8	72.7	3	27.3
Fluoroquinolone	Ciprofloxacin	7	63.6	4	36.4
	Ofloxacin	7	63.6	4	36.4
	Norfloxacin	5	45.5	6	54.6
Quinolones	Nalidixic acid	8	72.7	3	27.3
Folate pathway inhibitors	Cotrimoxazole	7	63.6	4	36.4
Phenicol	Chloramphenicol	0	0	11	100

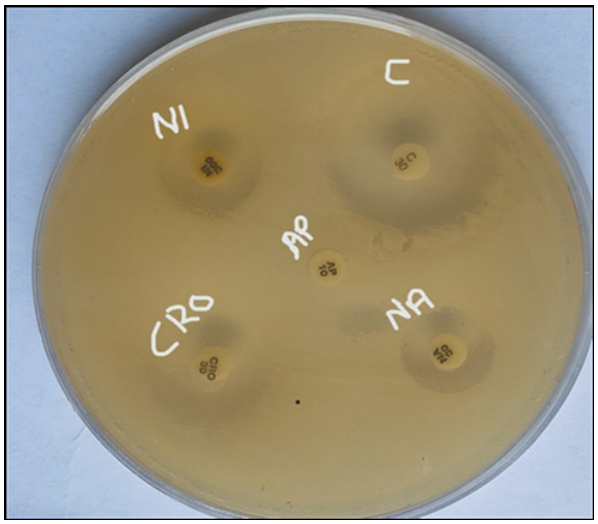

Figure 3: Antibiotics susceptibility pattern of *E. coli* (Chloramphenicol (S), Ceftriaxone (R), Nalidixic Acid (R), Ampicillin (R), Nitrofurantoin (R).

Antibiotic Susceptibility pattern of *K. pneumoniae:* *K. pneumoniae* was found to be most sensitive towards Chloramphenicol (100%). However, it was resistant to Ampicillin (100%) followed by Nitrofurantoin (100%) and Cefotaxime (75%) (Table 4).

Table 4: Antibiotic Susceptibility pattern of *K. pneumoniae* (N=4)

Groups of Antibiotics	Antibiotics used	Antibiotics used			
		Resistant		Susceptible	
		No.	%	No.	%
Penicillin	Ampicillin	4	100	0	0
ß-lactamase Inhibitor	Amoxycillin/Clavulanic acid	1	25	3	75
	Piperacillin/Tazobactam	1	25	3	75
Cephalosporin	Cefotaxime	3	75	1	25
	Cefixime	2	50	2	50
	Ceftriaxone	2	50	2	50
	Cefepime	1	33.3	2	66.7
	Ceftazidime	2	50	2	50
Cephalosporin	Imipenem	1	25	3	75
	Meropenem	1	25	3	75
Nitrofurans	Nitrofurantoin	4	100	0	0
Aminoglycosides	Gentamycin	0	0	4	100
	Amikacin	0	0	4	100
Tetracycline	Tetracycline	1	25	3	75
	Doxycycline	2	50	2	50
Fluoroquinolone	Ciprofloxacin	1	33	2	66.7
	Ofloxacin	0	0	4	100
	Norfloxacin	0	0	4	100
Quinolones	Nalidixic acid	2	50	2	50
Folate pathway inhibitors	Cotrimoxazole	2	50	2	50
Phenicol	Chloramphenicol	0	0	4	100

MDR among bacterial isolates: Out of 15 bacterial isolates, 93.3% were found to be MDR. Among them, 90.9% (10/11) of *E. coli* and 100% (4/4) of *K. pneumoniae* were MDR.

Zone of inhibition of plant against bacterial isolates: All of the plant extracts had shown antibacterial activities against the test organisms including *P. aeruginosa* and *Enterobacter* spp. isolates from pus. The higher

antibacterial activity of plant extracts was observed at the higher concentration (20mg/ml) as compared to the lower concentration (5mg/ml). Ethanolic extract of *C. asiatica* and *C. reflexa* demonstrated higher antibacterial activity against *E. coli* with zone of inhibition of 16 mm and 14 mm respectively. Likewise, the acetone extract of *C. reflexa* were effective against *K. pneumoniae* with zone of inhibition 14 mm (Table 5, Figure 4).

Table 5: Zone of inhibition of plant against Bacterial isolates (mm)

Bacterial pathogens	Different plant extracts	*Mentha spicata*			*Cuscuta reflexa*			*Centella asiatica*			Chloramphenicol
		5	10	20	5	10	20	5	10	20	
E. coli	Ethanol extract	0	10	13	7	10	14	8	12	16	26
	Acetone extract	7	7	9	0	10	11	9	12	15	
	D/W extract	0	0	6	0	0	8	6	7	8	
K. pneumoniae	Ethanol extract	0	8	10	0	8	10	8	10	12	27
	Acetone extract	0	6	8	0	8	14	8	10	12	
	D/W extract	0	6	8	0	6	9	7	9	11	
P. aeruginosa	Ethanol extract	0	0	7	0	6	9	6	11	13	16
	Acetone extract	0	0	7	0	6	8	0	6	8	
	D/W extract	0	0	0	0	0	7	6	8	10	
Enterobacter spp.	Ethanol extract	0	0	7	0	6	9	0	6	9	26
	Acetone extract	0	0	0	0	0	6	0	0	8	
	D/W extract	0	0	0	0	0	0	0	0	8	

***Note**: P. aeruginosa* and *Enterobacter* spp. were collected from pus samples

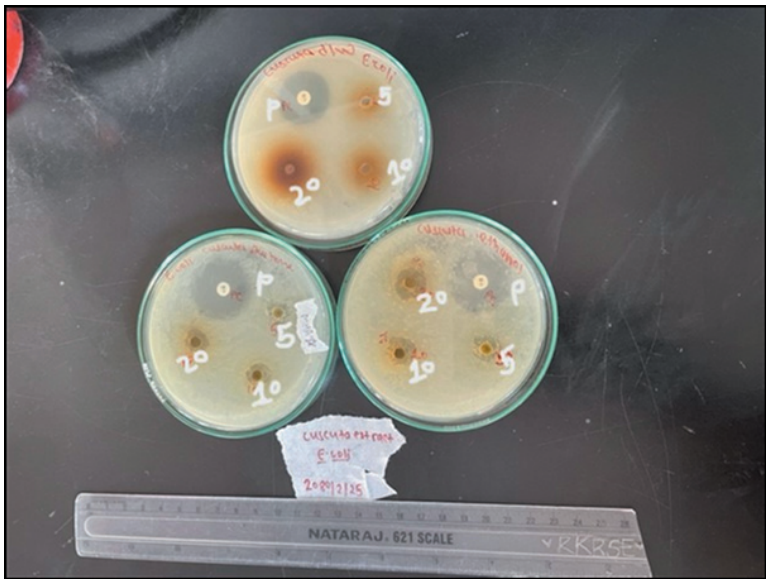

Figure 4: Antimicrobial activity of *Cuscuta reflexa* extract against *E. coli* by well diffusion method

MIC of plant extracts against bacterial isolates: MIC value of *C. asiatica* and *C. reflexa* against *E. coli* were found to be 5 mg/ml and acetone extract of *C. reflexa* was 10 mg/ml. *M. spicata* was found to be least effective against all clinical isolates (Table 6, Figure 5).

Table 6: MIC of plant extracts against bacterial isolates (mg/ml)

Bacterial pathogens	Different plant extracts	*Mentha spicata*				*Cuscuta reflexa*				*Centella asiatica*			
		5	10	20	MIC	5	10	20	MIC	5	10	20	MIC
E. coli	Ethanol extract	G	NG	NG	10	NG	NG	NG	5	NG	NG	NG	5
	Acetone extract	G	G	NG	20	G	NG	NG	10	G	NG	NG	10
	D/W extract	G	G	NG	20	G	G	G	_	G	G	NG	20
K. pneumoniae	Ethanol extract	G	G	NG	20	G	G	NG	20	G	G	NG	20
	Acetone extract	G	G	G	_	G	NG	NG	10	G	G	NG	20
	D/W extract	G	G	G	_	G	G	G	_	G	G	NG	20
P. aeruginosa	Ethanol extract	G	G	NG	20	G	G	G	_	G	NG	NG	10
	Acetone extract	G	G	G	_	G	G	G	_	G	G	G	_
	D/W extract	G	G	G	_	G	G	G	_	G	G	G	_
Enterobacter spp.	Ethanol extract	G	G	G	_	G	G	NG	20	G	G	NG	20
	Acetone extract	G	G	G	_	G	G	G	_	G	G	NG	20
	D/W extract	G	G	G	_	G	G	G	_	G	G	G	_

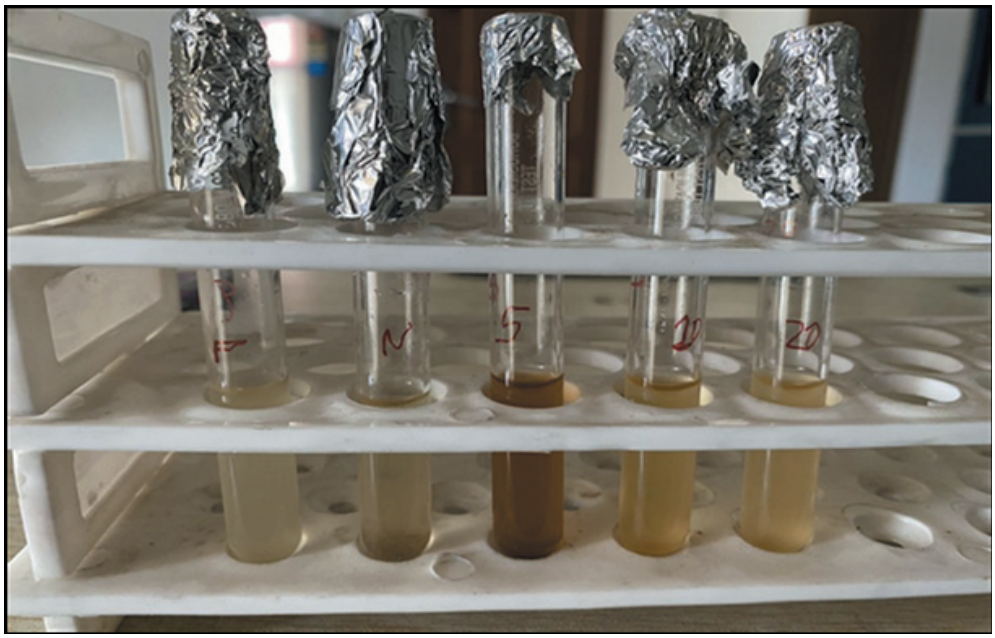

Figure 5: MIC of distilled water extract of *Mentha spicata* against *P. aeruginosa* by Broth tube dilution method (Growth: 5 mg/ml, 10mg/ml, 20mg/ml, P(positive), NG: N (Negative)

DISCUSSION

The current study provides two distinct information. One part of the study has been focused on the etiological profile and antibiotic susceptibility pattern of bacterial uropathogens while the second part evaluated the antimicrobial activity of 3 selected medicinal plants collected from local areas to different bacterial pathogens including multidrug resistant strains.

Out of 360 urine samples processed, the rate of bacterial UTI was found to be 4.2%. The study conducted by Acharya et al. (2011) showed a higher growth of 24.9%. Similar study conducted by Ganesh et al. (2019) also showed higher rate of UTI (12.3%) than our study. This variability in the rate of bacterial UTI depends on variation on patient's population, geographic location, hospital, ward/unit, or from patients who are under antibiotic treatment as found in the study conducted by Orenstein and Wong, (1999). Beside these, prior use of antibiotics by the patient before admission in

the healthcare facilities and the patient undergoing treatment within the health care facilities were likely to have lower growth rate in urine culture (Upadhyaya et al. 2013).

The rate of UTI was found higher in females (6.38%) in comparison to male (1.74%) in our study. Similar result has been reported by Magliano et al. (2012). In addition, Sood and Gupta (2012) reported even more higher rate of infections among the female patients accounting for 62.42% in females and 37.67% in males. The difference in gender wise distribution of UTIs might be due to anatomical differences between the male and female urethras, inadequate perineum cleaning, the use of napkins, sanitary towels, and tampons, along with pregnancy and sexual activity which is supported by the result of the study conducted by Hooton et al. (1996). We also observed slightly higher rate of infection among the age group ranging from NB-19 i.e.,7.5%. The main causes of childhood UTIs may be due to cultural practice, prolonged use of diaper, lack of proper sanitation, etc. Nowadays, UTI is main problem in children. Pulipati et al. (2017) reported that UTIs are more common in female children due to congenital anomalies, anatomical abnormalities in urologic functions, and vesico-uteral reflux in females. Elderly man is mostly infected by UTI similar to study of KC et al. (2012), this might be due to increase in the prevalence of diabetes mellitus and male prostate illness, respectively as found in the study conducted by Mahesh et al. (2010).

The present study showed a higher rate of UTI among outpatients 13(4.38) in comparison to 2(3.17) inpatients, which is not similar to the study conducted by Patel et al. (2012) reported that 30.23% of the samples were positive from OPD and 51.26% positive from indoor. The rapid growth rate of UTI in outpatients indicates that the infection might be community acquired.

Among the total positive cases, all the isolates were gram negative (100%). Similar findings have been reported by Faryabi et al. (2014), where 96.7% of the isolates were gram negative. The study conducted by Prakash *et al*. (2011) showed 90.32% Gram Negative bacteria which is high in number in comparison to gram positive bacteria. Members of Enterobacteriaceae have been well described as the primary agents for UTI than other organisms in several studies. Among the Gram's negative organisms, *E. coli* was predominant isolates (73.33%) which was similar to the studies conducted by Malmartel and Ghasarossian (2016) and Baral et al. (2012) reporting 73% and 81.3% of *E. coli* isolates respectively. Likewise, Ganesh et al. (2019) reported 57.8% *E. coli* isolates from UTI patients in Nepal. The reason for the predominance of *E. coli* might be due to it is released in the feces, inoculating the periurethral region or vagina, and then being introduced into the urinary system during periods of physical manipulation, such as during sexual activity or catheterization as found in the study conducted by Hooton et al. (1996).

All *E. coli* isolates were found to be sensitive to Chloramphenicol followed by Tetracycline (72.7%). In a similar by Kibret and Abera, (2011), 72.6% isolates were susceptible towards Tetracycline. However, in this study, the least effective drugs for treatment of UTI caused by *E. coli* were Cefotaxime and Cefixime (9.1%). Thus, *E. coli* are the bacteria most frequently implicated in uncomplicated UTI and catheter-associated UTI and are becoming increasingly resistant to antibiotics. The antibiotic susceptibility of *K. pneumoniae* showed that the isolates were 100% sensitive towards chloramphenicol followed by Ofloxacin, Norfloxacin, Gentamycin and Amikacin. In the same way, 100% were resistant to Ampicillin followed by Nitrofurantoin.

Out of 15 uropathogens, 14 (93.3%) were multidrug resistant. A study conducted in 2019 reported 62% of *E. coli* and *K. pneumoniae* as MDR strains among hospitalized patients in Nepal (Ganesh et al. 2019). The probable explanation for the higher rate of MDR and antibiotic resistance, especially in developing countries like Nepal may be attributable to irregular antibiotic prescription, genetic geographic, social behaviors and sampling biases and different patient`s characteristics as found in the study conducted by (Moini et al. 2015). The fact that there are so many MDR bacteria involved in the development of UTIs is quite dangerous and cannot be disregarded. Therefore, vital actions by relevant authorities and politicians are needed to quickly control this issue.

Many studies collectively suggest that *Centella asiatica* extracts have antimicrobial activity against various pathogenic bacteria. This study coincides with the study of Dash et al. (2011) which found that ethanol extracts of *Centella asiatica* exhibited higher antimicrobial activity against bacteria compared to

water extracts. Jagtap et al. (2009) also reported that the ethanolic extract of *Centella asiatica* showed higher antimicrobial activity than water extracts. According to Wulansari et al. (2023) by using 100% concentrated infusion from gotu kola leaves, the highest average hindrance of 9.67 ± 0.36 mm and 10.12 ± 1.12 mm against *E. coli* and *S. aureus* was accomplished which is slightly lesser than our study. This difference might be due to the concentration of plants extract used and experiment method used. Jacob and Shenbagaraman, (2011) did not find any antimicrobial activity of water and ethanol extracts of *C. asiatica* against *E. coli* and *K. pneumoniae*. In the study by Senthilkumar (2018), *C. asiatica* was highly effective against *E. coli* (20.6 mm), *K. pneumoniae* (16.2 mm) *and P. aeruginosa* (19.2 mm) at 250 µl concentration. This difference may be due to geographic region, method followed, climatic condition, soil chemistry, environmental condition of place in which plant cultivated

Akarsh and Thippeswamy (2020) found antimicrobial activity of ethanolic extract of *Cuscuta reflexa* against *E. coli, K. pneumoniae, and Pseudomonas aeruginosa* with 19 mm, 22 mm and 23 mm zone of inhibitions at 100 mg/ml and 15 mm, 16 mm and 16 mm at 25 mg/ml concentrations respectively. Our results showed slightly harmonious with the results above at 25 mg/ml concentration. The present study result is similar with the results of Dhanalakshmi et al. (2018) who reported acetone extract of *C. asiatica* did not show significant activity against *Enterobacter* spp. while ethanol extract of *C. asiatica* showed significant growth with 7 mm zone of inhibition at 30µg/disc. Similarly, the extract of *M. spicata* exhibited least antibacterial effect against the bacterial pathogens which is similar with the study by Shahbazi, (2015). He showed that Gram-positive bacteria were more susceptible to *M. spicata* essential oil than Gram-negative bacteria. Furthermore, we observed an effective antimicrobial activity of acetone extract of *C. reflexa* while little or no previous study was found to compare with our results. Despite the fact that a number of extracts demonstrated good antibacterial potency, the commercial use of these plants for treatment of UTIs have not been reported yet.

CONCLUSIONS

This study reported an emergence of increasing drug resistance among bacterial pathogens from different clinical samples. The situation of drug resistance is an alarming to empirical therapy for MDR associated infections. As an alternative to antibiotics for treating MDR strains, we found that the crude plant extracts, particularly the acetone and ethanol extracts, had a considerable amount of efficacy against the bacterial pathogens. In addition, these traditionally used medicinal plants and their antimicrobial extracts could be the novel drugs that target the primary therapeutic requirements.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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