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Natural Phenolics Exhibit Strong Antimicrobial activity against Clinical Isolates Obtained from Patients of Urinary Tract Infection

Shahbaz Alam[1], Neelam Singh[2*], Nashra Afaq[3] and Chayanika Putatunda[1]

[1]Department of Microbiology, Om Sterling Global University, NH-52 Hisar- Chandigarh Road Hisar, Haryana- 125001, India
[2]Department of Microbiology, Saaii College of Medical Science and Technology, Kanpur- 209203, India
[3]Rama Medical College Hospital & Research Centre, Mandhana, Kanpur, India

**Corresponding author*

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A B S T R A C T

A Comprehensive study of the Antimicrobial efficacy of Gallic acid and Tannic acid on the clinical isolates of the UTI microflora as a promising component. This was a Cross sectional study carried out in the Department of Microbiology at Om Sterling Global University, Hisar, Haryana, India for a period of 1 year i.e, between June 2022 to June 2023 upon pathogenic bacteria from urine samples of UTI patients visiting the New Reliable Path labs, Qurban Road, Chandwara, Muzaffarpur (Bihar), India. A total of 350 freshly voided midstream urine samples from the people whose initial routine urine tests were positive for pus cells and albumin were collected in a sterile wide mouth container. The Gallic acid (CAS No 5995-86-8, product code 27645) and tannic acid (CAS No. 1401-55-4, product code 403040) standards were purchased from Merck, St. Louis, MO, USA where the stock solutions of both gallic acid and tannic acid were prepared by dissolving 50 mg of the compound in 1 mL of phosphate-buffered saline (PBS) containing 10% DMSO, to determine the minimum inhibitory concentration. The MICs and MBCs were determined performing broth dilution. In the present study it was observed that the tannic acid (T.A.) was more effective as compared to the gallic acid (G.A.). The MIC value of 225±1.06 was observed for T.A. and 1750±18.66 for G.A. against *E coli*. The lowest MIC of tannic acid was observed for *Staphylococcus saprophyticus* 90±1.06 followed by *Acinetobacter baumannii* with 125±3.14 and highest for *Proteus vulgaris*375±6.14. In case of gallic acid the lowest MIC was recorded for *Pseudomonas aeruginosa* with 600±8.66 followed by *citrobacter freundii* with 750 ±3.14 and the maximum was observed for *Klebsiella pneumoniae* observed 2050 ±1.21. Tannic acid can serve as a promising candidate for treating (multi drug resistant) MDR Urinary Tract Infections. It was also found that the drugs with lower MIC scores are more effective antimicrobial agents.

Introduction

Urinary tract infections are the most common type of community acquired as well as commonest among nosocomial infections. These are a group of infections that occur in the urinary tract and hence known as Urinary tract infections (UTIs) which may affect any part of the urinary tract. It causes more financial burden on society. Commonly occurring UTI is cystitis, which occur in the bladder, where urine is stored. Other UTIs involve the kidneys (pyelonephritis), bladder (cystitis), prostate (prostatitis), urethra (urethritis) or urine (bacteriuria). These are common bacterial infections affecting humans throughout their life span (Steven *et al.,* 2006 and Kucheria *et al.,* 2005). UTI is the third most common cause of admission to hospitals in India. According to estimates about 6 million patients per year visit healthcare clinics worldwide for UTI out of which around 30,000 are treated in the wards (Bano *et al.,* 2012). UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteraemia in hospitalized patients (Weinstein *et al.,* 1997; Stamm, 2001; Gastmeier, 2001). UTI also varies based on severity (i.e., complicated versus uncomplicated). Complicated UTI is the infections in urinary tract with structural or functional abnormalities or the presence of foreign objects (eg. placing urethral catheter). There is also a practice of categorizing UTI into first infection and recurrent infection. Recurrent infections are again subdivided into unresolved bacteriuria, bacterial persistence, and reinfection. Pain, fever, and discomfort are the common manifestations due to the urinary tract infections and it can be easily treated.

Treatment becomes tough when the infection spreads to the kidneys (Hvidberg *et al.,* 2000). The impact of UTIs on individuals is significant, as infections negatively affect individuals' mental health and sense of well-being Grigoryan *et al.,* (2022). Antibiotics are usually prescribed to kill the bacteria causing the infection. Therapeutic agents like antimicrobials may not function properly because of noncompliance, deficiency in absorption, improper drug metabolism, and uropathogens becoming resistant and unresponsive to the therapy provided.

This can be cured altering the therapy according to antimicrobial sensitivity tests carried out by a proper urine culture. Bacterial persistence and reinfection occur when the route cause of infection in the urinary tract is not eradicated. The uropathogens escape for the antimicrobial therapy by hiding themselves where the therapeutic agents cannot reach like urinary calculi and foreign objects (Steven *et al.,* 2006).

The majority of UTIs develop in the normal urinary tract and are therefore termed 'uncomplicated'. Symptoms of lower urinary tract infection include high urination frequency, painful urgency and haematuria (haemorrhagic cystitis). Other causes of symptoms of lower tract irritation will need to be considered. It is customary to separate sexually transmitted disease from UTI on the basis of pathogens and site of infection and yet the distinction between urethritis, vaginitis and cystitis may be very in distinct symptomatically. Moreover, pathogens which are more familiar populating the urethra and genitalia, e.g. Chlamydia, urea plasma, gardnerella and other unfamiliar or fastidious organisms may cause cystitis but are otherwise undetected by standard urine culture techniques. Nevertheless, they have a variable contribution to statistics on epidemiology of UTI (Engel and Schaeffer, 1998). Prominent symptoms include painful urination, frequent urge to urinate, cloudy or strong-smelling urine, pelvic pain (in women), rectal pain (in men). In severe cases, fever, chills, and nausea can occur. A key symptom of lower Urinary tract infection remains frequent urination along with the feeling of having tour in ate even though there may be very little urine to pass. Other less common symptoms are Nocturia (need to urinate during the night), Urethritis (discomfort or pain at the urethral meatus or a burning sensation throughout the urethra at the time of urination (dysuria). Pain may be felt in the midline suprapubic region accompanied with Pyuria (pus in the urine or discharge from the urethra), Hematuria (Blood in urine), Pyrexia (Mild fever), cloudy and foul-smelling urine etc. Increased confusion and associated falls are common presentations to Emergency Departments for elderly patients with UTI. Some urinary tract infections are a symptomatic. Protein may also be found in the urine. For kidney infections all of the above symptoms could be shown. Emesis (Vomiting is common). Acute back, side (flank) or groin pain, abdominal pain or pressure with shaking chills and high spiking fever, night sweats, extreme fatigue may be encountered in a few cases.

Pathogens responsible for community acquired cystitis and pyelonephritis comprise 70% *E.coli* and the remainder a variable contribution from *Proteus, Klebsiella, Enterococcus and Pseudomonas*. In the

summer and autumn *Staphylococcuss aprophyticus* is a relatively common isolate on culture (5–10%) in young adult females. Somewhat different proportions of organisms are found in isolates of urine from patients treated in hospitals and institutions.

The differences reflect the greater incidence of complicated Colitovaginal and buccal mucosa in women susceptible to UTI (Fowler *et al.,* 1977; Schaeffer *et al.,* 2002; Bergamin *et al.,* 2017). Antimicrobial resistance surveillance is necessary to determine the size of problem and to guide empirical selection of antimicrobial agents for treating infected patients.

Urinary tract infections are common among women. It affects one in five women during their life time (Foxman, 2002). UTIs are not as common in men under the age of 50, but they are prone to complications like stone or enlarged prostate. About 20 % of women who have one infection will have a recurrence. Of this group, 30 % will have a third occurrence, and of this group, 80 % have additional recurrences. It is mostly due to antibiotic resistance (Head, 2008). UTIs are common during pregnancy period. About 8% of pregnant women experience with UTI (Delzell and Lefrvre, 2000). Increased bacterial population in vagina is the main reason for UTI in both pregnant (Sharami *et al.,* 2007) and non pregnant women (Harmanli *et al.,* 2000). UTI may spread to kidney during pregnancy due to the part of urethral dilation and hydronephrosis.

Sexual activity, using a diaphragm or spermicide, menopause, and certain medical conditions can also increase the risk of developing a UTI. Drinking plenty of water and avoiding irritants like caffeine, alcohol, and spicy foods can also help alleviate symptoms. Prevention may be done by executing urination before and after sexual activity, wipe front to back after using the toilet, wear cotton underwear and loose-fitting pants, drink plenty of water, and avoid irritating feminine products (Moore *et al.,* 2008).

This is a frequent travellers' infection who accidentally get it via public toilet use. Apart from being common infection described in outpatient setting and hospital patients it is widespread throughout the patient care units including outpatient setting and hospital patients. (Shaifali *et al.,* 2023). Among the in-patients of large tertiary care units who stay for many days within the wards this healthcare-associated infection (HAI) sets in easily and secondly in the kidney stone patients who undergo lithotripsy and have urethral stents implanted or catheters introduced via their external genitalia it is prevalent.

UTIs are further classified according to the presence of predisposing conditions for infection (uncomplicated or complicated) or the nature of the event (primary or recurrent). (Li *et al.,* 2022). Antimicrobial Resistance Collaborators (2022); Sihra *et al.,* (2018). Usually infection is caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus saprophyticus* or *Enterococcus faecalis*, while the hospital acquired ones are *Escherichia coli, Pseudomonas aeruginosa*, Proteus sp, Enterobacter sp., *Serratia* sp. or Enterococcus.

Uropathogenic *Escherichia coli* (UPEC) is the most common causative agent for both uUTIs and cUTIs, followed by other pathogenic microorganisms, such as *Klebsiella pneumoniae*, *Proteus mirabilis, Enterococcus faecalis,* and *Staphylococcus* spp (Patel *et al.,* 2019). The incidence of UTI among women rises by 5% per decade. There is an increase in UTI during pregnancy by about 7% and untreated bacteriuria may lead to UTI in 25% cases (Habak and Griggs, 2023).

Urinary tract Infection (UTI) is among the most. In both community and hospital settings the family which is on the top of the list among those responsible for UTIs is Enterobacteriaceae while the predominantly isolated pathogen is uropathogenic *Escherichia coli* (UPEC) (Mohapatra *et al.,* 2022). The latter is also the most common causative agent of cUTI (Bader *et al.,* 2020). In almost all cases empirical antimicrobial treatment initiates before the laboratory results of urine culture are available; thus antibiotic resistance may increase in uropathogens due to frequent use of antibiotics. Children and elderly individuals may present with atypical symptoms of UTIs, making diagnosis and treatment challenging. In addition, individuals with underlying medical conditions, such as diabetes or a weakened immune system, are at higher risk for developing complicated UTIs.

Ongoing research is focused on developing new treatments for UTIs and improving our understanding of the mechanisms behind recurrent infections. There are several beneficial effects reported for gallic acid, and the tannic acid including antioxidant, anti-inflammatory, and antineoplastic properties. The ability of some plant secondary metabolites to act as resistance-modifying

agents is a promising field in mitigating the spread of bacterial resistance. The goal of this study was to determine the current prevalence of urinary tract infection and the find out a solution to tackle the urinary tract infection causing microbes using plant derived natural drugs.

Materials and Methods

The study carried out in the Microbiology section at New Reliable Path labs, Qurban Road, Chandwara, Muzaffarpur (Bihar), India for a period of one year i.e, between June 2022 to June 2023. Our study recruited people who appeared to be in good condition and were open to taking part. Patients with any other diseases and those who had not provided their consent for the trial were also excluded from it. Samples were taken from 250 individuals falling in the age group 20 -40 years. Freshly voided midstream urine samples from the patients whose initial routine urine tests were positive for pus cells and albumin were collected in a sterile wide mouth container. All urine samples were processed within an hour of collection for aerobic bacterial culture. If samples were delayed, they were refrigerated at 4°C and processed within 4 to 6 hours.

Microscopic Study

One of the diagnosis criteria of UTI was based on microscopic findings of more than 10 pus cells/ high power field (40×) in urine were included in the study. Centrifugation at 1500 RPM was done for 20 min and urine sediment was utilised for direct microscopic examination of red blood cells (RBCs), leukocytes, epithelial cell, casts, crystals, and parasites. In the normal urine sediment, a few count of RBCs, pus cells (0–5/high power field), and epithelial cells may be found. Epithelial cell count were reported as "few," "moderate," or "many" per low-power (10X) field.

Culturing of urine samples and Characterization of Bacteria

Significant bacteriuria was defined as culture of a single bacterial species from the urine sample at a concentration of more than 100,000 cfu/ml. Using a calibrated loop, a urine sample was inoculated onto a standard culture media called Cystine-Lactose- Electrolyte-Deficient (CLED) agar. For 18 hours, culture plates were incubated in an ambient air incubator at 35–37°C. The culture plates were examined for the presence of bacterial colonies after the allotted time was over. Using the colony count method, their growth was classified as significant or not. By growing isolated colonies on various media, such as MacConkeys agar and blood agar, they were further described based on cultural traits (Qiao *et al.,* 2013). Further, the isolates were identified by cultural, morphological and biochemical tests. The method used in the identification and characterisation of isolated bacteria included Gram staining, motility test and biochemical tests like, TSI and IMViC according to Cheesbrough (2002); Cheesbrough (2004).

Antibiotic susceptibility

Antibiotics used- Ceftriaxone, Ceferperazine, ofloxacin, Gentamicin, Cefiximine, Levofloxacin, Amikacin, Carbapenems, Doxycline. Interpretation of results was done using the zone sizes. The zone of inhibition of greater than 10 mm was considered sensitive, 5–10 mm as moderately sensitive and <10 mm or no zone of inhibition as resistant (CLSI, 2016; CLSI 2022).

Antimicrobial activity of phenolics towards UTI pathogenic bacteria

All reagents were of the best commercial grade available and used without further purification. Tannin standards 1-*O*-galloyl-β-D-glucose and penta-*O*-galloyl-β-D-glucose were purchased from Merks (Milan, Italy). Ethanol, cyclohexane and dimethyl sulfoxide were analytical grade solvents obtained from Merks, Fluka (Milan, Italy).

Preparation of the Phenolics (gallic acid and tannic acid solutions)

Gallic acid (CAS No 5995-86-8, product code 27645) and tannic acid (CAS No. 1401-55-4, product code 403040) standards were purchased from Merck, St. Louis, MO, USA. Cellulose acetate filters were obtained from Hi Media, India, Bacto agar was acquired from Hi Media, India, Dimethyl sulfoxide (DMSO) was Merk's, St. Louis, MO, USA.

Polyphenol solutions

Stock solutions of both gallic acid and tannic acid were prepared by dissolving 50 mg of them in 1 mL of phosphate-buffered saline (PBS) containing 10% DMSO,

as described elsewhere (Díaz-Gomez *et al.,* 2013). Both stock solutions were sterilized by filtration through cellulose acetate filters (0.2 mm pore size; 25 mm diameter).

Bacterial strain and growth conditions

The bacteria used in this study were taken from freshly grown broth cultures, overnight incubated. They were recovered and routinely grown for 24 h at 37°C in Luria-Bertani (LB) broth (Sambrook *et al.,* 1989). UTI pathpgens growth assay in liquid medium: Cell suspensions composed of 3 x 10^7 cells were transferred into tubes containing 5 mL of LB supplemented with a range of concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 mg/mL) of gallic acid or tannic acid. After incubation with constant reciprocating motion (250 rpm) at 37 -C for 24 h, bacterial growth was determined by turbidimetry at 600 nm (Stevens *et al.,* 1991) or by counting colony forming units per ml(CFU/mL).

Minimal inhibitory concentration (MIC) determination

The antibacterial activity of the tannic acid and the gallic acid was determined using sterile 2ml 96-well plates The 12 wells of each row were filled with 0.5 ml sterilized Mueller Hinton agar. Sequentially, wells 2–11 received an additional 0.5 ml of a mixture of culture medium and extract of different dilution of gallic acid and tannic acid serially diluted to create a concentration sequence where the two hundred milligrams of the test compound (tannic acid or gallic acid) was added to 0.4 ml of dimethyl sulfoxide DMSO to obtain a concentration of 500 mg/ml.

Then, 100 µl was used containing 250 mg. Thereafter, there was a serial dilution of the extract in each tube to obtain concentrations of 125, 62.5, 31.25, 15.62, and 7.81 mg ml^{-1}, respectively. One hundred microliters of 10^6 CFU/ml of each of the tested bacterium were pipetted out into each test tube and incubated at 37 °C for 24 h. Two control tubes were used: nutrient broth inoculated with bacteria and no antibiotic or no plant extract or any of gallic acid or tannic acid was used as a positive control and nutrient broth containing no inoculum was used as a negative control. The lowest concentration that kills the organisms completely, where no bacterial growth is observed (MBC) CLSI (2016), was determined by assaying the test tubes resulting from MIC determinations. A loopful of the content of each test tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37 °C for 24 h and observed for bacterial growth (Usman *et al.,* 2014).

Results and Discussion

Out of 250 urine samples received in the Microbiology Laboratory at New Reliable Path labs, 90 (36%) urine samples showed significant growth for UTI. The ratio of females 55 (61.1%) were more as compared to that of the males 35 (38.9%) (Table no. 1). The age group of 21-30 (47.7%)was affected the most followed by 31-40 years (24.4%) of age and least in the age group above 61 years of age (2.2%) (Table no.2).

Urine infections are the most common type of bacterial infections in developing countries including India. Gillam *et al.,* (2023) of UNC Medical Centre, in the latest Carolina Antimicrobial Stewardship Program Guideline have stated that in a urine culture with bacterial count > 100k CFU/ml the likelihood of UTI must be investigated, further treatment guided and diagnostic workup initiated. Gender wise variation is reported in almost all the studies performed so far with females outnumbering males (Janifer *et al.,* 2009). Our results are also similar wherein 61.2% of females suffered from UTI where as the fraction of males was 38.8%. In our investigation the maximum number of cases (47%) were in the age group 21-30 years which could be because the young individuals are more conscious about infections and readily consult the medical practitioners. We did our sampling by collecting the midstream urine as for past few decades, mid-stream urino-culture has been considered the gold standard for UTI diagnosis. However, in about one-third of cases, a positive culture is not obtained, and it has become increasingly clear that bacteria may be present in the healthy bladder (Andolfi, 2020). In addition, patients with recurrent UTI due to treatment failure caused by antimicrobial-resistant strains have a reduced quality of life. In addition, because overuse of antibiotics is a major factor in the development of MDR bacteria and because about 25 percent of all antibiotic prescriptions are for UTIs, antibiotic prophylaxis should be used once all nonantibiotic treatment options have been exhausted (Antibiotic resistance collaborators, 2022). The most recommended nonantibiotic prevention and treatment options for recurrent UTIs include cranberries, intravaginal probiotics (*L. rhamnosus, L. reuteri*), D-mannose, hippurate methenamine, estrogen-releasing vaginal ring in postmenopausal women, and immunostimulants.

Table.1 Gender wise distribution of the UTI cases

Gender	Total no. of Cases studies (N=90)	Percentage
Male	35	38.8 %
Female	55	61%

Table.2 Age wise distribution of the UTI cases

S. No.	Age (in years)	No. of Cases	Percentage
1.	0- 10	0	00.0
2.	11-20	11	12.2 %
3.	21-30	43	47.7 %
4.	31-40	22	24.4%
5.	41-50	9	10%
6.	51-60	3	3.3%
7.	≥61	2	2.2%

Table.3 The Frequency of bacteria isolated from the UTI cases

Type of Organism Isolated	No. of Isolates	Percentage
Escherichia coli	44	48.8%
Klebsiella pneumoniae	17	18.8%
Proteus mirabilis	8	8.8%
Pseudomonas aeruginosa	4	4.4%
Citrobacter freundii	6	6.6%
Klebsiella oxytoca	2	2.2%
Proteus vulgaris	1	1.1%
Acinetobacter baumannii	1	1.1%
Enterococcus faecalis	2	2.2%
Staphylococcus saprophyticus	1	1.1%
Staphylococcus aureus	4	4.4%
Total	**90**	**100%**

Image.1 Urine Samples

Table.4 Biochemical Characters of bacterial Isolates from UTI samples

Bacteria	Gram staining	Lactose utilization	TSI	Starch Hydrolysis	Lipid Hy	Casein	Catalase	Oxidase	Nitrate Reduction	indole	MR	VP	Citrate utilization	Urease
E.coli	-, rods	+	A	-	-	+	+	-	+	+	+	-	-	-
Klebsiella pneumoniae	-, rods	+	A	-	-	-	+	-	+	-	+	+	+	+
Proteus mirabilis	-, rods	-	A	-	-	-	-	-	+	+	+	-	-	+
Pseudomonas aeruginosa	-, rods	-	NC	+	-	-	+	+	+	-	+	-	+	-
Citrobacter freundii	-, rods	-	AG	-	-	-	+	-	+	-	+	-	+	-
Klebsiella oxytoca	-, rods	+	A	-	-	-	+	-	+	+	-	+	+	+
Proteus vulgaris	-, rods	-	AG	-	-	-	+	-	+	+	+	-	+	+
Acinetobacter baumannii	-, short rods	+	K	-	-	-	+	-	-	-	-	-	+	-
Enterococcus faecalis	-, rods	+		-	-	-	-	-	+	-	-	+	-	-
Staphylococcus saprophyticus	+, rods	-	AG	-	-	-	-	-	-	+	-	-	-	-
Staphylococcus aureus	+, cocci	+	AG	-	-	-	-	-	-	+	-	-	-	-

NC= No Change; A= Acid slant; G= Gas Production, K = Alkaline slant

Table.5 Antibiotic Sensitivity Profile of the UTI Pathogens

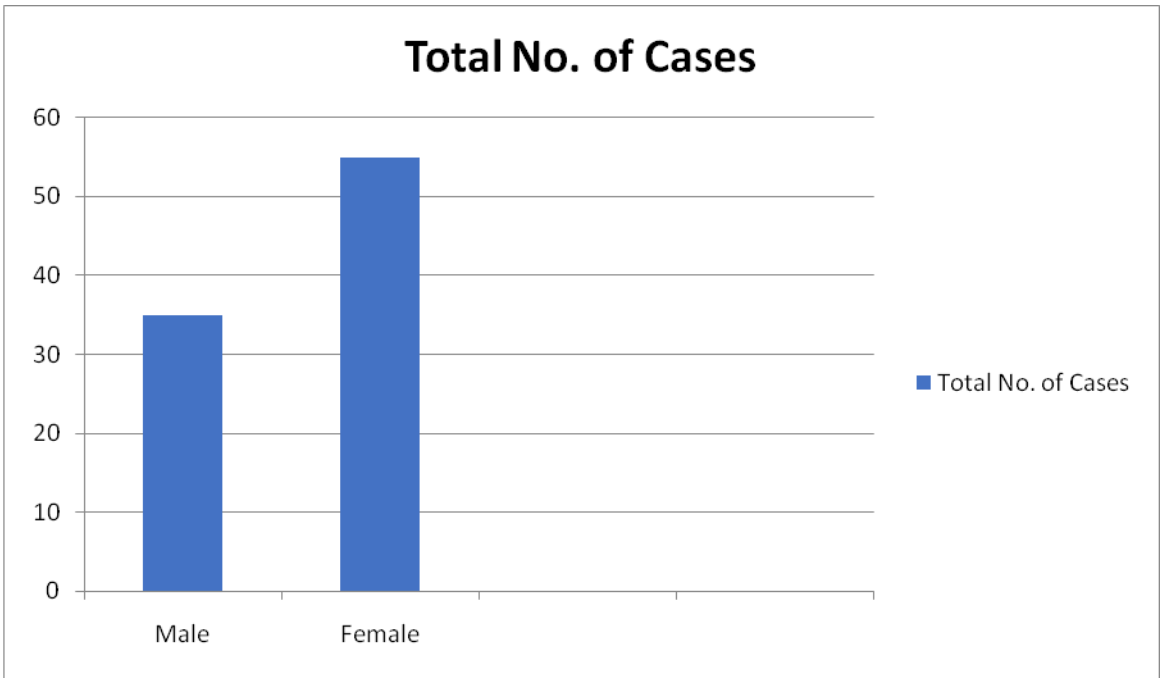
Isolate	Antibiotic									
	CF	CP	OF	CM	GM	LN	AK	CA	DX	PX
E.coli	S	I	S	I	S	R	S	R	I	S
Klebsiella pneumoniae	I	S	S	S	S	I	I	S	I	I
Proteus mirabilis	S	S	I	I	S	I	S/R	R	I	S
Pseudomonas aeruginosa	I	S	I	R	S	I	S	S	S	S
Citrobacter freundii	R	R	I	R	S	I	S	S	I	I
Klebsiella oxytoca	R	S	R	S	R	R	I	R	S	S
Proteus vulgaris	S	I	S	R	S	S	I	I	I	I
Acinetobacter baumannii	R	R	I	S	I	S	R	S/R	S	I
Enterococcus faecalis	S	R	S	I	S	S	S/R	I	S	S
Staphylococcus saprophyticus	R	S	S	S	I	S	I	R	S/R	R
Staphylococcus aureus	R	S	S	S	I	I	I	R	S	R

Abbreviations for Antibiotics: Ceftriaxone = CF, Cefoperazone= CP, Ofloxacin = OF, Gentamicin =GM, Cefixime = CM, Levofloxacin = LN, Amikacin = AK, Carbapenem=CA, Doxycycline=DX, Polycycline= PX

Table.6 Antibacterial Effect of Gallic acid and Tannic Acid showing Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) concentration.

Pathogenic Bacteria	MIC (µg/ml)		MBC (µg/ml)	
	G.A.	T.A.	G.A.	T.A.
Escherichia coli	1750	225	4000	64
Klebsiella pneumoniae	2050	300	325	32
Proteus mirabilis	975	250	550	32
Pseudomonas aeruginosa	600	175	500	40
Citrobacter freundii	750	150	300	40
Klebsiella oxytoca	1750	275	425	64
Proteus vulgaris	850	375	275	80
Acinetobacter baumannii	1250	125	300	32
Enterococcus faecalis	1200	160	350	48
Staphylococcus saprophyticus	1400	90	5000	32
Staphylococcus aureus	1575	128	5500	40

Graph.1 Graphical representation of Gender wise distribution of the UTI cases



In this observed *E.coli* to be predominant UTI causative agent (48.8 %) which is in accordance to previous reports by other research groups (Lee *et al.,* 2018; Kang *et al.,* 2018). *Klebsiella pneumoniae* is the second most common pathogen seen here (18.8%). *Proteus vulgaris*, *Staphylococcus aureus, Enterococcus faecalis, Staphylococcus saprophyticus*, and *Acinetobacter baumannii* were also encountered in low frequencies by is in agreement with other reports worldwide (Hunt *et al.,* 2023). Drug resistance is a prevailing and ever aggravating phenomenon among bacteria occurring in UTI samples (Paul, 2018; Didelot and Pouwels, 2019; Yelin, 2019). It was also observed by us. A significantly high degree of drug resistance among bacterial isolates was observed. Our study showed a very high rate of resistance (>70%) among *E. coli* isolates to piperacillin. Among *Klebsiella* isolates, no resistance was found for meropenem and low resistance was found for ciprofloxacin (5.8%), norfloxacin (5.8%), and cefotaxime (23.52%) but high for nitrofurantoin (94.15%) and trimethoprim/sulfamethoxazole (41.17%). Piperacillin resistance has also been reported in *E.coli* and other related genera of Gram negative bacteria (Erb *et al.,* 2007; Rashid *et al.,* 2014). Drug resistance in *Klebsiella* has been studied and published by a large number of medical researchers (Li *et al.,* 2022). The antimicrobial properties of pure phenolic compounds and polyphenols of some types of alcoholic drinks (wines) for UTI pathogens were carried out by Rodrigues *et al.,* (2007) and it was reported that bacterial species exhibited different sensitivities towards the phenolic compounds. *Escherichia coli* was the most sensitive bacterium and Flavobacterium sp. was resistant against all phenolic compounds tested. (Rodrigues *et al.,* 2007). Phenolics present in plants are well known for their antimicrobial activity and gallic acid and tannic acid act as a promising component inhibiting the growth of human pathogens. Tannic acid has been shown more effective as an antimicrobial by scientists across the world (Wang *et al.,* 2022). *Klebsiella pneumoniae* is largely inhibited by gallic and vanillic acids as well as quercetin.

According to Rodrigues *et al.,* (2007), gallic acid was more effective than other phenolics tested against *Klebsiella pneumoniae* achieving a strong antimicrobial activity from 500 mg/l. Staphylococcus aureus ATCC 25923 was resistant to all pure phenolic acids (Rodrigues *et al.,* 2007). The MDR strains of *E.coli*, *S. aureus*, *Streptococcus bovis, Pseudimonas aeruginosa, Salmonella typhimurium, Escherichia coli, Klebsiella pneumoniae* and *Candida albicans* are usually sensitive to the antimicrobial activity of many plant extracts comprising mainly tannins. These plants viz. *Acacia*

nilotica, *Syzygium aromaticum* and *Cinnamum zeylanicum* were demonstrated to have strong inhibitory potential towards UTI pathogens mentioned above. (Khan *et al.,* 2009) which is in agreement with our findings.

The future of the antimicrobial resistance control is uncertain, the strategy of association of drugs with new substances is one of the most promising factors. Therefore, as we can see from the present study that the compounds present themselves as substances that can be promising potentiating agents of antimicrobial drugs.

Author Contribution

Shahbaz Alam: Investigation, formal analysis, writing—original draft. Neelam Singh: Validation, methodology, writing—reviewing. Nashra Afaq:—Formal analysis, writing—review and editing. Chayanika Putatunda: Investigation, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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