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Evaluation of antibacterial activity of pomegranate (*Punica granatum* L. *and* Citrus lemon against urinary tract infection causing bacteria *E. Coli, Enterococcus fecalis, Enterobacter aerogenes* collected from various diagnostic laboratories of Khwazakhela, swat

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Abstract

E. Coli is one of the major gram-negative pathogens well known causative agent for almost all kind of UTIs, however Enterobacteriaceae such as *Enterobacter aerogenes* and *Enterococci fecalis* are responsible for UT infections. The present study was conducted to evaluate the *in vitro* antibacterial activity of ethanol and aqueous extract of pomegranate and lemon peels against (23 isolated stains) *E. Coli*, (16 isolated strains) *Enterococcus faecalis* and (9 isolated strain) of *Enterobactor aerogenes* recovered from the urine sample of patientsin Swat KPK-Pakistan. The Agar dilution and well method was applied for the antibacterial activity of pomegranate and lemon peelextracts. All of the three subject isolates were exposed to different concentrations of pomegranate and lemon peel. MIC of aqua extract of pomegranateagainst the subject isolates of *E. Coli* was 200 µl/mLand ethanol extract was 50 µl/mL. So the ethanol extract of pomegranate was found to be more effective as compared to the aqueous extract. On the other hand MIC of aqua extract lemon peel against the subject isolates of *E. coli* was100 µl/mL and ethanol extract was 25 µl/mL. So the ethanol extract of pomegranate was found to be more effective as compared to the aqueous extract. MIC of ethanol extract of pomegranate against the isolates of *Enterococcus fecalis* was found smaller (50 µl/mL) than that of water extract (100 µl/mL). So the ethanol extract of pomegranate was found to be more effective as compared to the aqueous extract. On the other hand. MIC of aqua lemon extract against the subject isolates of *Enterococcus fecalis* was 100 µl/mL and ethanol extract was 25 µl/mL. Mainly the ethanolic extract of lemon peel was more effective than the ethanolic extract of pomegranate against *Enterococcus fecalis* isolates.

The MIC of the water and ethanolic extract of pomegranate against *Enterobacter aerogenes* was found 500 µl/mL and 200 µl/mL respectively. So the ethanol extract of pomegranate was found to be more effective as compared to the aqueous extract. On the other hand MIC of the ethanolic extract of lemon peel was 100 µl/mL, while the water extracts showed inhibition against the *Enterobacter aerogenes* isolates at the concentration of 250 µl/mL. So the ethanol extract of lemon was found to be more effective as compared to the aqueous extract.For the determination of antibacterial activity of both pomegranate and lemon extracts (in ethanol and water) method as described in Clinical and Laboratory Standard Guidelines (CLSI) was adopted. The subject isolate of *E. Coli*, *Enterococcus fecali*, and *Enterobacter aerogenes* were shown different sensitivities (sensitive >10mm zone inhibition or resistant <10mm zone inhibition t) for ciprofloxacin with a variable zone of inhibition.

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Keywords: *E. Coli*; *Enterobacter aerogenes; Enterococcifecalis*; Pomegranate and lemon; Antibacterial activity; Urinary tract infection

1. Introduction

E. Coli well known causative agent for almost all kind of UTIs, however Entero bacteriaceae such as K. pneumoniae, P. mirabilis, Citrobacter, Enterobacter, and other bacteria for instance *P. aeruginosa, A. baumannii, S. aureus, Staph. saprophiticus, E. faecalis, S. bovis,* and *C. albicansare* responsible for UT infections [1] . UPEC are responsible for around 80% of uncomplicated UTIs (95% of community related, and 50% of hospital-received UTI). UPEC similarly, the supreme common causative agents in complex UTIs as well [2] .

The microbial resistance to antibiotics has made herbal medication more popular [3] . It was reported that lemon peel extracts are highly effective against *E. Coli*isolates[4]. Comparative studies on antimicrobial resistance description and genomic animus features of *E. Coli* isolates from human UT, farmed animals predominantly chicken presented a great resemblance [5]. The other pathogens e.g. *Klebsiella, Shigella,* and *S. aureus* were also recovered from different patients, but the frequency was very low [6]. Therefore, these three pathogens were included in this study to examine the antibacterial efficacy of lemon peel and pomegranate peel extract. *E. Coli* and *E. faecalisis* are responsible for the majority of urinary tract infections in Punjab, Pakistan [7]. According to this study, these pathogens were resistant to majorities of antibiotics like cephalexin, cephradine, pipemidic acid, amikacin, nalidixic acid, amoxicillin/clavulanic acid, ampicillin and aztreonam.

Studies were commenced in various regions of the world on anti-bacterial potential of various extracts of the pomegranate and its plant parts against a wide range of different bacteria, using disc diffusion methods or MIC methods [8]. Work conducted on anti-bacteria effect of peel, bark and fruits of pomegranate against the human health affecting bacteria presented in Table 1.

Table 1 Anti-bacterial potential of pomegranate parts against the human health influencing bacteria

Microbial strain	**Part/extract**	**Ref.**
L. monocytogenes	Peel, dried juice powder	[9][10][11]
A. baumannii	Peel	[12]
A. actinomycetemcomitans	Peel	[13]
B. breve	BPG	[14]
B. infantis	BPG	14]
Bifidobacterium spp.	BPG	14]
Clostridium spp.	BPG	14]
E. Coli	Peel,bark	[9,10.11[
E. Coli	Peel	[10][11][12]
H. pylori	Peel	[18]
K. pneumonia	Peel	[10];[11] ;[12]
Lactobacillus spp.	BPG	[14]
P. gingivalis	Peel	[13]
Proteus spp.	Peel	[19]
P. aeruginosa	Peel, flower extract	[10]; [11];[12]
S. aureus	Peel	[20][21]2005;[22]; [23];[24][25]
S. aureus	Peel, juice, and BPG	[26]; [27];[28]
S. aureus	Peel	[29]

S. entericaserovars	Peel	[10]
S. epidermidis	Peel	[29]
S. Typhi	Peel	[30]; [31]
S. Typhimurium	Peel	[26]
S. Anatum	Peel	[10]; [11]; [12]
S. Typhimurium	Peel	[10]; [11]; [12]
Sh. Sonnei	Peel	[26]
Shigella spp.	Peel	[32]; [33]
S. aureus	Peel	[10];[11]; [12]
S. mitis	Peel	[34]
S. mutans	Peel	[29], ;[34].
S. pneumoniae	Peel	[10];[11].,; [12]
S. salivarius	Peel	[29]
S. sanguis	Peel	[34]
V. cholera	Peel	[32]
Yersinia enterocolitica	Peel	[9]

*BPG: By products of pomegranate

The results of said study on antibacterial advantages of various pomegranate extracts counter to clinically based dental bacteria. No reliable results were reported against the urinary tract pathogens. But nevertheless, it was discovered that the peel extract worked well against *E. Coli* [35].

Citrus fruits are a rich source of flavanones and many polymethoxylated flavones, which are found very rare in other plants, have very strong antibacterial activity [36][37][38][39]. By using the disc diffusion method [40] demonstrated that a lemons peel ethanolic extract exhibits exceptional antibacterial efficacy when used against *E. Coli*. With an inhibition zone of 18.77mm, the ethanolic extract of the lemon peel was found to have 100% antibacterial activity against *E. Coli*. [41] showed similar outcomes for Algerian lemon extract against several drug-resistant microorganisms. The reported inhibition zone for different lemon extracts against *E. Coli* was 12 mm. Similar results were reported by [42] on the 20 mm zone of inhibition against *E. Coli* from peel extracts of Algerian lemon. These kind of products can be preferred as antibiotic resistance of available antibiotic against bacteria is serious issue. So, therefore always be in search of substitute medicines [43] .

2. Material and methods

2.1. Pomegranate and Lemon Sample Collection and Handling

The pomegranate and lemon peels were collected from local juice vendors situated at different location in Swat (34°-40′ to 35° N latitude and 72′ to 74°-6′ E longitude). Select only un-rotten and fresh peel sample. Fresh fruits of pomegranate and lemon were also procured from local market, washed with tape water and then unpeeled manually with hands.

2.2. Peel Processing

Both kinds of collected peels were air dried in an open air shade for few days and then partially dried samples were brought to the laboratory. Air dried sample was further dried in oven at 60 ^{o}C for 24 h to remove the maximum moisture content. Oven dried samples were grinded in order to make a fine powder with minimum particle size.

2.3. Preparation of Ethanol Extract

Ethanol extract preparation was achieved by soaking 20 gm powder (both pomegranate and lemon) in 100 ml of ethanol in 250 ml conical flask, mixed with glass rod. Flask was kept in dark for 24 hours at room temperature. Decant and

collected the upper ethanol layer in 500 ml flask. Added 100 ml of 70% ethanol another time in sample residue and thoroughly mixed. Decant and pooled the both extracts upper layer in 500 ml flask. Collective extracts were filtered through Whatman filter paper and transferred in round bottom flask of rotatory evaporator. Evaporated the alcoholic extract till dryness at 50°C under an appropriate vacuum pressure. The dried residue was dissolve in 10 ml of DMSO and transferred to a test tube. Final extract was stored at freezing temperature. Before application for their antibacterial activity extract was filtered via 0.45 μm syringe filter for the sterilization purpose.

2.4. Preparation of Water Extract

Weighed 20 g of dried peels powder in a 250 ml flask. Added 100 ml of deionized water and mixed well thoroughly. Placed the flask in thermostatic water bath shaker at 5 °C for 30 minutes. The Liquid extract was centrifuged at 2000 rpm for 10 minutes and the supernatant was transferred to a 100 ml flask. De ionized water was added to make the final volume 100 ml water extract was filtered by means of 0.45 µm syringe filter for the sterilization.

2.5. Preparation of Stock Extracts

The concentration of both ethanolic/water extracts was adjusted up to 1000 mg/mL. The working dilutions were made using the stock solution in the range of 10-1000 µg/mL.

2.6. Study Population, Urine Sample Collection and Isolation of Bacteria

The study population is based on patients complaining urinary tract infection attending two clinical laboratories of (Ameerk Clinical Laboratory and Anwar Clinical Laboratory) Khwazakhela Swat. The samples were procured under the accord of subjected patients. The study population included male, female and children of all age groups. The urine sample (≈ 25 mL) were collected from patients in sterile sampling containers and processed for microbiological analysis. Briefly, a loop full from the sampling container was streaked on Tryptone Soya Agar, Nutrient Agar, and Mac Conkey's Agar plates and incubated at 37°C for 24h. After 24 h incubation period individual colonies were selected and identified on the basis of biochemical characteristics.

2.7. Isolation of *E. Coli*, Entero bacteria eruginosa & Enterococcous species in urine sample

The typical colonies from Mac Conkey's agar were collected and streaked on Eosin Methylene blue agar. The plates were incubated at 37 °C for 24 h. The green metallic sheen indicated the presence of *E. Coli*.

A single colony was picked from Tryptone Soya Agar and streaked on CLED AGAR and incubate at 37°C for 24 h. Next Day growth was observed; the lactose fermenter (Enterobacter) produced pink colonies. The *Enterococcus* produce yellow color pin pointed pink colonies on the CLED plate. The Gram staining was performed for further confirmation.

Pick the isolated colonies of LF and inoculated in a set biochemical test tube (TSI triple sugar iron, citrate, urea, SIM sulphiteindole motility). For the *Enterococcous* pick the isolated colonies and emulsify in the tryptone broth for the antibiotic sensitivity and inoculate the isolated *Enterococococcus* species in the 6.5% NaCl and bile-esculin. From the tryptone broth lawing on the MH mullerhilton agar for LF. From the tryptone broth lawing on the SBA agar for *Enterococcous*. Placed the antibiotic disc on the lawing plates and incubate the plates and biochemical test tube at 37°C for 24 hours for Enterobacter. Placed the antibiotics disc on the lawing plate of SBA and 6.5% NaCl and bile-esculin and incubate at Carbondioxide incubator for *Enterococcous*.

2.8. Oxidase Test

A single colony was picked and inoculated on Nutrient agar plate. It was incubated at 37°C for 24 h.After 24 h purity was confirmed and colonies were spread on oxidase strip. No change in oxidase strip color indicated oxidase negative. Strip color converted into purple indicated oxidase positive. The *E. Coli* was used as negative control and *P. aeruginosa* was positive control. The identified *E. Coli* (23 stains), *Enterococcus faecalis* in (16 strains) and 9 isolated strain of *Enterobactor aerogenes* were inoculated in TSA broth (in sterilized 10 mL screw capped test tubes) and immediately shifted to PCSIR Labs Karachi for sensitivity studied.

2.9. Preparation of Inoculums

The subject isolates of *E. Coli, Enterococcus fecalis* and *Enterobacter aerogenes* were inoculated into Muller Hinton broth augmented with 5% de-fibrinated sheep blood and incubated for 18 h at 35°C. The bacterial count in fresh MHB was adjusted up to 3.0×10^8 CFU/mL in accordance with to Macfarland standards.

2.10. Agar Diffusion Method

For the determination of antibacterial activity of pomegranate and lemon extracts (in ethanol and water) method as described in Clinical and Laboratory Standard Guidelines was adopted. Concisely, a 500 μL (~10^8 CFU/mL) of subject was smeared on MH agar containing 5% sheep blood (defibrinated). Using a sterilized tube 7mm wells were prepared on agar plate, subsequent to a 15 minutes of inoculation. Each wells (of independent experiment) were filled with a 15 μL ethanolic extracts. As control the disks of ciprofloxacin antibiotics were also laid on agar surface. The prepared plates were incubated initially at 4°C for 1 h and then placed at 35°C for 24 h. The antibacterial effect of the extract was assessed by measuring the size of the clear zone of inhibition. The zone of inhibition of antibiotic disk was considered as control.

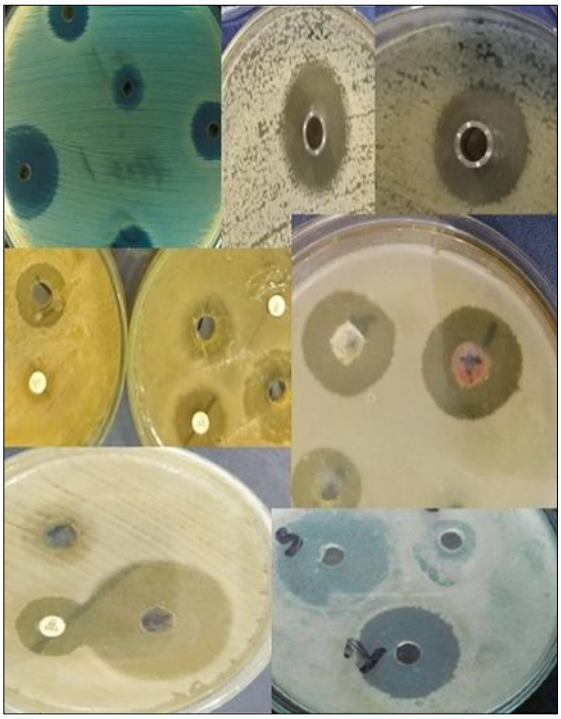

Figure 1 Some representative pictures showing zone of inhibition of pomegranates and lemon peel extract effect on isolates show prominent zone of inhibition

3. Results

Total of 108 samples were collected for bacterial isolation. The samples were then inoculated on several medium, such as Tryptone Soya Agar, Eosine Methylene Blue Agar, MacConkey's Agar, and Eosine Methylene Blue Agar. After inoculation plates were incubated at 37 °C for 24 h. The next day plates were observed for bacterial growth and colonies were picked for identification.

On MacConkey's agar and EMB agar, 108 samples out of them revealed a presence of *E. Coli*in a 21.3% rate. The EMB agar was used for further confirmation of *E. Coli*. The colonies with green metallic sheen were confirmed as *E. Coli*. These isolates were purified and preserved in 16% glycerol broth at -20°C. The CLED media showed the presence of *Enterococcus faecalis*in 14.8% samples and *Enterobactor aerogenes* in 8.3% samples. The lactose fermenter *Enterobactor* produces yellow color pin pointed colonies on CLED agar plate.

3.1. Antibacterial effect of pomegranates and lemon extracts against the subject isolates of *E. Coli*

Twenty-three subject isolates of *E. Coli* were exposed to different concentrations of pomegranates and lemonpeel extract via micro-dilution agar in tryptone soya broth. For this purpose, water extract and ethanol extracts of pomegranates and lemon peel were applied to designate the minimum inhibition concentration which shows clear zone of inhibition Fig.**1**.

Table 2 MIC of pomegranate and lemon peel extracts against the subject isolates of *E. Coli*

S#	Gander	Age	PomegranatePeel		Lemon Peel		Antibiotic Sensitivity
			Water Extract	Ethanolic Extract	Water Extract	Ethanolic Extract	Ciprofloxacin
1	M	25	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
2	M	13	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
3	M	41	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
4	M	22	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
5	M	24	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
6	M	29	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
7	M	48	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
8	M	56	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
9	M	08	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
10	M	37	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
11	M	23	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
12	M	42	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
13	M	11	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
14	M	49	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
15	M	11	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
16	M	15	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
17	M	8	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
18	M	11	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
19	M	59	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
20	M	7	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
21	M	29	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
22	F	23	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
23	F	67	200 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R

Note = S: sensitive (>10mm zone of inhibition); R: resistant (<10mm zone of inhibition); M; male; F: female

The MIC of pomegranate and lemon peel extracts against the subject isolates of *E. Coli* is presented in Table **2**. The results showed that all of the isolates were inhibited at the MIC of 50 µl/mL. On contrary that the water extract MIC was 200 µl/mL. Likewise, the ethanolic extract of lemon peel extract was more effective than that of its water extract. The MIC of the ethanolic extract of lemon peel against the *E. Coli* isolate was 25 µl/mL while the MIC of its water extract was found 100 µl/mL against the subject isolates for UTI patients.

3.2. Antibacterial effect of pomegranates and lemon extracts against the isolates of Enterococcus fecalis

The MIC of both extracts of pomegranates/ lemon against the isolates of *Enterococcus fecalis* is presented in Table 3. Thirteen isolates of *Enterococcus fecalis* were tested for the antibacterial activity and all were found sensitive to different MIC of ethanoic and water extracts of both respective peels of pomegranate and lemon. The MIC of ethanolic extract of pomegranate against the isolates of *Enterococcus fecalis* was found smaller (50 µl/mL) than that of water extract (100 µl/mL).

The ethanolic lemon peel extract was also found smaller (25 µl/mL) than its corresponding water extract 100 µl/mL. The results show that mainly the ethanolic extract of lemon peel was more effective than the ethanolic extract of pomegranate against *Enterococcus fecalis* isolates. In order to see the control effect ciprofloxacin was tested. The subject isolates of *Enterococcus fecalis* were dissimilarly sensitivities.

Table 3 MIC of pomegranate/ lemon peel extracts against the subject isolates of *Enterococcus fecalis*

S#	Gander	Age	Pomegranate Peel		Lemon Peel		Antibiotic Sensitivity
			Water Extract	Ethanolic Extract	Water Extract	Ethanolic Extract	Ciprofloxacin
1	M	22	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
2	M	37	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
3	F	61	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
4	M	23	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
5	F	37	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
6	F	24	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
7	F	31	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
8	M	57	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
9	M	45	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
10	F	27	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
11	M	30	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
12	F	17	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
13	F	23	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R
14	F	07	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
15	M	52	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	S
16	M	64	100 µl/mL	50 µl/mL	100 µl/mL	25 µl/mL	R

Note = S: sensitive (>10mm zone of inhibition); R: resistant (<10mm zone of inhibition); M; male; F: female

3.3. Antibacterial effect of pomegranates/ lemon extracts against the isolates *Enterobacter aerogenes*

Nine *Enterobacter aerogenes* strains were isolated from the urine of UTI patients. The antibacterial effect of pomegranate and lemon peel extracts were studied the MICs against the subject isolates. The resulting MICs of both peel extracts (pomegranate and lemon) against *Enterobacter aerogenes*are presented in Table 4.

The water extract from pomegranate was found less effective than that of its ethanol extract. The MIC of the water/ethanolic extract of pomegranate against nine subject isolates of *Enterobacter aerogenes* was found 500 µl/mL and 200 µl/mL respectively. The lemon peel extracts were found comparatively more effective that the extracts of pomegranate. The MIC of the ethanolic extract of lemon peel was 100 µl/mL, while the water extracts showed inhibition against the *Enterobacter aerogenes* isolates at the concentration of 250 µl/mL. The control effect of ciprofloxacin was also tested against these isolated strains. The subject isolate of *Enterobacter aerogenes* were shown different sensitivities for ciprofloxacin with a range zone of inhibition.

Table 4 MIC of Pomegranate/ lemon peel extracts against the *Enterobacter aerogenes* isolates

S#	Gander	Age	PomegranatePeel		Lemon Peel		Antibiotic Sensitivity
			Water Extract	Ethanolic Extract	Water Extract	Ethanolic Extract	Ciprofloxacin
1	F	43	500 µl/mL	200 µl/mL	250 µl/mL	100 µl/mL	S
2	M	41	500 µl/mL	200 µl/mL	250 µl/mL	100 µl/mL	R
3	M	59	500 µl/mL	200 µl/mL	250 µl/mL	100 µl/mL	S
4	M	33	500 µl/mL	200 µl/mL	250 µl/mL	100 µl/mL	R
5	F	34	500 µl/mL	200 µl/mL	250 µl/mL	100 µl/mL	S
6	M	35	500 µl/mL	200 µl/mL	250 µl/mL	100 µl/mL	S
7	M	39	500 µl/mL	200 µl/mL	250 µl/mL	100 µl/mL	R
8	F	21	500 µl/mL	200 µl/mL	250 µl/mL	100 µl/mL	R
9	F	7	500 µl/mL	200 µl/mL	250 µl/mL	100 µl/mL	S

Note = S: sensitive (>10mm zone of inhibition); R: resistant (<10mm zone of inhibition); M; male; F: female

The results indicated that these extracts are highly effective against major pathogens e.g. *E. Coli, Enterobacter* and *enterococci* responsible for urinary tract infections in Swat.

4. Discussion

The gram negative pathogens are the most frequent cause of urinary tract infections [44]. According to a recent report around 150 million people are annually infected by gram negative bacteria. In the current study, most of the isolates were found gram negative *E. Coil,* (21 isolates) *Enterobacter aerogenes*(13 isolates), however, only less number gram positive (*Enterobacter aerogenes*) isolates were identified in the urine samples of the studied area. The studies have reported that around 80% of urinary tract infections are caused by gram negative bacteria and about 15 to 20% are caused by gram positive pathogens.

Majority of *E. Coli* and *Enterococcus* species recovered with urinary tract infections were resistant to 1st and 2nd line antibiotics[45]. Uncomplicated cases of urinary tract infections are very common in women of all groups across Pakistan [46][47, 48]. Some plants e.g pomegranate (*Punicagranatum* L.), black chokeberry and cornelian cherry have great potential for the prevention of UTI. Nearly every part of these plants have been tested for antimicrobial activities [49][50][51]. The high efficacy of ethanol extract from lemon peel is associated with the presence of a high quantity of flavanones and many polymethoxylated flavones [39][40][41][52] .

The pomegranate contains a high quantity of punicalagin compound responsible for antimicrobial activity. Consumption of these compounds as fruits or their extract is beneficial for the control of infectious diseases [53]. Lemon and pomegranate are also rich sources of vitamin C and consumption of these fruits boosts the immune system and prevents bacterial growth inside the body [54]. Vitamin C is mainly suggested as a supplement for prevention of recurrent UTI [55]. In addition to this, it is also reported that vitamin C prevents bacterial adhesion to the wall of the urinary tract and to indwelling catheters [56] **.** The consumption of these fruits induces the immune system, and thus controls bacterial infections [57][58][59]. Overall, in this study, PP-EE and LP-EE have shown prominently greater antibacterial activities against UTI isolate than that of their corresponding water extracts. In a recent report, it has been proved that the methanolic extract of pomegranate contains a high quantity of Ellagic acid bioactive tannin. This compound was highly effective against biofilm-positive strains of *S. aureus* and *E. Coli* [60, 61]. Similarly, the ethanol extract from lemon peel contains a high quantity of flavonoid that can inhibit specific enzymes and scavenge free radicals and has also shown an eradicating effect against *E. Coli*. [45].

In addition to this, the lemon peel also contains compounds that may disrupt the cell membrane and cell wall of gram negative and positive bacteria [62]. In the present study, the highest activity was observed for ethanol extracts. This suggested that the majority of the antibacterial compounds in lemon and pomegranate peel are ethanol soluble. *Enterobacter* was more tolerant to these compounds as compared to *E. Coli* and *Enterococci*. The *E. Coli* isolates were

comparatively sensitive to these extracts. Interestingly, the ethanol extract of lemon peel and pomegranate peel was more effective against all three isolates.

5. Conclusion

Khwazakhela is an administrative district Tehsil in the Khyber Pakhtunkhwa province's Swat. Urinary tract infections are one of the major health issue of this region. Instead of having good options for folklore medicinal sources, they prefer to rely on allopathic treatment. The current study was part of such kind of endeavor in order to search the new medicinal substitute to cure urinary tract infections.

In the present study total of (23 isolated stains) *E. Coli*, (16 isolated strains) *Enterococcus faecalis* and (9 isolated strain) of *Enterobactor aerogenes* recovered from the urine sample of patients in Swat KPK-Pakistan.

The high prevalence of multidrug-resistant pathogens further complicated the situation.Amoxicillin-clavulanic acid, Ciprofloxacin, and Trimethoprim-sulfamethoxazol are currently applicable antibiotic practice for the management of UT infections. The overall resistance of these antibiotic was reported to be 48% to 85% in under developing countries in compassion to developed countries 3.1- 37.1%.

The present study was carried out in Khwazakhela Swat region of KPK Pakistan. According to the study conducted by Anis-ur-Rehman the incidence and clinical contour of UTI in children of Hazara Division Pakistan was not considerably dissimilar from that of developing and developed countries of the world.

The water extract from pomegranate was found less effective than that of its corresponding ethanolic extract. The lemon peel extracts were found comparatively more effective then the extracts of pomegranate. The subject isolate of *Enterobacter aerogenes* were shown different sensitivities for ciprofloxacin with a range zone of inhibition. This study has proved that pomegranate and lemon peel are the best examples of this. However, the results of the present study confirmed that pomegranate and lemon peel are very effective against a wide range of isolates of *E.coli* recovered from the urine samples of patients in Swat.

Compliance with ethical standards

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Disclosure of conflict of interest

This manuscript has no conflict of interest with any party

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