Original Article

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Microbiology Section

Evaluating Antibacterial Efficacy of Extract of *Punica granatum* against ESBL and Carbapenemase Producing Multidrug Resistant Uropathogenic *Escherichia coli*: An In-vitro Study

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ABSTRACT

Introduction: Uropathogenic *Escherichia coli* (UPEC) is the primary causative agent of Urinary Tract Infections (UTIs) and has shown a concerning rise in Multidrug Resistance (MDR), including the production of Extended-Spectrum Beta-Lactamases (ESBLs) and carbapenemases. This resistance compromises the efficacy of conventional antibiotics, posing serious clinical threats such as pyelonephritis and urosepsis, in both community- and hospital-acquired settings.

Aim: To evaluate the in-vitro antibacterial activity of *Punica granatum* extract against MDR Uropathogenic *E. coli*, including ESBL- and carbapenemase-producing MDR UPEC isolates.

Materials and Methods: This in-vitro study was conducted at Acharya Vinoba Bhave Rural Hospital (AVBRH), Jawaharlal Nehru Medical College (JNMC), Wardha, Maharashtra, India, over a period of seven months from May 2023 to November 2023. A total of 273 non duplicate UPEC isolates were included. These isolates were obtained from symptomatic UTI patients of all age groups and both sexes who provided clean-catch midstream urine samples during the study period. Identification of UPEC isolates was performed using standard biochemical methods. Phenotypic detection of ESBL and carbapenemase production was carried out in accordance with Clinical and Laboratory Standards Institute (CLSI) 2022 guidelines. Molecular characterisation involved Polymerase Chain Reaction (PCR) amplification for the detection of CTX-M genes (ESBL) and NDM genes (carbapenemase). The antibacterial activity of *Punica granatum* extract, prepared in different concentrations, was assessed using the Kirby-Bauer disc diffusion method. Mean zones of inhibition were compared using Student's t-test, and a p-value of <0.05 was considered as statistically significant.

Results: Out of 273 UPEC isolates, 192 (70.3%) were identified as MDR. Among these, 82 were ESBL producers and 62 were carbapenemase producers, while 48 isolates were non producers of both ESBL and carbapenemase enzymes. The *P. granatum* extract demonstrated significant antibacterial activity, with mean zones of inhibition of 21.18±1.4 mm against ESBL-producing UPEC and 21.54±0.93 mm against carbapenemase-producing UPEC at 100% concentration, comparable to gentamicin. The antibacterial activity of the extract was dose-dependent, with minimal effect observed at lower concentrations. Solvent controls showed negligible antibacterial activity, confirming the specificity of the extract.

Conclusion: The study highlights a high prevalence of MDR UPEC, particularly among hospitalised patients, underscoring the urgent need for alternative therapeutic strategies. The significant antibacterial activity of *Punica granatum* extract against ESBL- and carbapenemase-producing UPEC suggests its potential as a natural, plant-based adjunct or alternative to conventional antibiotics in the treatment of UTIs, especially in resource-limited settings.

Keywords: Antibacterial agent, Extended-spectrum beta-lactamases, Plant extract, Urinary tract infection

INTRODUCTION

The UPEC is the predominant causative agent of UTIs and has increasingly acquired MDR, complicating treatment and increasing the risk of severe clinical outcomes such as pyelonephritis, sepsis, recurrent infections, and therapeutic failure [1]. Recent research has highlighted the antibacterial efficacy of *Punica granatum* (pomegranate) against MDR UPEC. This activity is attributed to its rich composition of bioactive phytochemicals, particularly polyphenols such as punicalagin and granatin B. These compounds inhibit bacterial growth and enhance the effectiveness of conventional antibiotics, positioning *P. granatum* as a promising natural alternative in combating antibiotic resistance [2].

Granatin B has demonstrated the highest binding affinity to MDR *E. coli* targets in molecular docking studies [3], whereas punicalagin functions as a sensitising agent that enhances antibiotic activity against resistant strains [4]. Furthermore, *P. granatum* has shown the ability to deactivate ESBL enzymes—key mediators of resistance in *E. coli*—thereby restoring the effectiveness of beta-lactam antibiotics [5]. Notably, *P. granatum* exhibits synergistic activity with antibiotics such as lincomycin and tetracycline, leading to a significant reduction in their Minimum Inhibitory Concentration (MIC) [6]. This synergism is critical for improving treatment strategies against resistant infections. Additionally, its antioxidant and anti-inflammatory properties contribute to enhanced infection management and recovery [7]. Given its diverse array of bioactive compounds, *P. granatum* holds substantial promise for the development of novel antimicrobial agents targeting ESBL- and carbapenemase-producing UPEC. Its potential use in combination therapy with existing antibiotics may offer more effective approaches for treating MDR infections.

Therefore, this work reinforces the significance of *P. granatum* as a promising candidate for the development of plant-based antimicrobials. The findings not only suggest its capacity to combat MDR UPEC but also highlight the importance of exploring herbal remedies as complementary strategies for addressing the global challenge of antibiotic resistance. While the initial results are encouraging, further investigations are necessary to elucidate the underlying mechanisms of action, assess safety and efficacy in clinical settings, and optimise its integration into mainstream treatment protocols. This study is novel in its comprehensive design, combining clinically isolated MDR UPEC strains with molecular detection of ESBL (CTX-M) and carbapenemase (NDM) genes. It uniquely evaluates the dose-dependent antibacterial activity of *P. granatum* extract against these highly resistant strains. By integrating molecular confirmation, systematic in-vitro testing, and robust statistical analysis, this study translates phytochemical potential into practical evidence supporting *P. granatum* as a potential natural alternative therapy.

Objectives

Primary objective: To assess the in-vitro antibacterial efficacy of *Punica granatum* extract against MDR UPEC isolates confirmed phenotypically and genotypically as ESBL- and carbapenemase producers.

Secondary objective: To compare the antibacterial activity of *P. granatum* extract with gentamicin and evaluate the association between inhibition zones, extract concentration, resistance gene presence (CTX-M and NDM), and patient demographic characteristics.

MATERIALS AND METHODS

The present in-vitro study was conducted to investigate antibacterial activity of *Punica granatum* extract against UPEC isolates producing ESBL and carbapenemases. The study was carried out from May 2023 to November 2023 at Acharya Vinoba Bhave Rural Hospital (AVBRH), Jawaharlal Nehru Medical College (JNMC), Wardha, Maharashtra, India. The study protocol was approved by the Institutional Ethics Committee (IEC) of Datta Meghe Institute of Medical Sciences (Deemed to be University), Wardha, Maharashtra, India (Ref. No.: DMIMS (DU)/IEC/2022/02 dated 15 July 2022). Written informed consent was obtained from all participants prior to enrollment.

Sample size: A total of 429 inpatients and outpatients presenting with symptoms of UTI were recruited during the study period. Among these, 273 urine cultures were positive for *Escherichia coli* and were included in the study.

The sample size was determined based on the reported prevalence of MDR UPEC in similar studies, ensuring adequate statistical power to detect differences in antibacterial activity [8]. Previous studies reported MDR rates of 61% among outpatients and 68% among inpatients in Isfahan, Iran [9], and 41.3% MDR with 64.3% ESBL production among pediatric complicated UTI cases in Nepal [10].

Inclusion criteria:

UPEC isolates obtained from symptomatic UTI patients of any age and sex. Patients who provided clean-catch midstream urine samples during the study period.

Exclusion criteria:

Duplicate isolates from the same patient. Contaminated or insufficient samples, defined as:

- Mixed growth of three or more different organisms in culture.
- Presence of non uropathogenic commensals such as *Lactobacillus* spp., *Corynebacterium* spp., and coagulase-negative staphylococci (except *Staphylococcus saprophyticus*).
- Samples showing heavy epithelial cell contamination on microscopy, signifying poor collection technique.

Study Procedure

A total of 273 UPEC strains were isolated from symptomatic UTI patients, including 146 females and 127 males. Clean-catch midstream urine samples (approximately 15 mL) were collected and transported to the Microbiology Laboratory at JNMC, Wardha, for further processing. Urine samples were cultured on MacConkey agar and 5% blood agar plates and incubated aerobically at 37°C for 18-24 hours. Suspected *E. coli* colonies were purified and maintained on nutrient agar slants. Identification of *E. coli* was performed based on standard cultural characteristics and biochemical tests. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, in accordance with Clinical and Laboratory Standards Institute (CLSI) 2022 guidelines [11].

Screening of *E. coli* for ESBL and Carbapenemase Production

Based on CLSI 2022 guidelines [11], ESBL production in *E. coli* isolates was initially screened using the combined disc method. Isolates exhibiting resistance to imipenem and meropenem were further screened for carbapenemase production.

Phenotypic confirmation of ESBL producing *E. coli*: Phenotypic confirmation of ESBL production was carried out using the Modified Double Disc Synergy Test (MDDST). In this method, a disc containing amoxicillin-clavulanate (20/10 µg) was placed at the centre of a Mueller-Hinton agar plate inoculated with the test isolate. Discs of third-generation cephalosporins (ceftriaxone, cefotaxime, and cefpodoxime) were placed at a distance of 15 mm from the central disc, while a disc of the fourth-generation cephalosporin (cefepime) was placed at a distance of 20 mm. An enhanced zone of inhibition extending toward the amoxicillin-clavulanate disc was interpreted as positive for ESBL production.

Phenotypic confirmation of carbapenemase producing *E. coli*: Phenotypic confirmation of carbapenemase production was performed using the Modified Hodge Test (MHT). Confirmed isolates were inoculated into 1 mL of tryptic soy broth and incubated overnight. The cultures were then preserved at –20°C with the addition of 20% glycerol for subsequent molecular analysis.

Plasmid DNA extraction for molecular analysis: Plasmid DNA extraction from ESBL- and carbapenemase-producing *E. coli* isolates was performed using the HiPurA® Genomic DNA Purification Kit (HiMedia, Mumbai, India), following the manufacturer's instructions. Briefly, 1.5 mL of overnight-grown bacterial culture was centrifuged at 13,000 rpm for 2 minutes at 25±2°C. The resulting pellet was resuspended in 180 µL of lysis solution, followed by the addition of 20 µL of Proteinase K and incubation at 55°C for 30 minutes. Subsequently, 20 µL of RNase solution was added, and the mixture was incubated at 25±2°C for 5 minutes. An additional 200 µL of lysis solution was then added, vortexed, and incubated at 55°C for 10 minutes. DNA precipitation was achieved by adding 200 µL of 95% ethanol, followed by vortexing. The lysate was transferred to a HiElute Miniprep spin column and centrifuged at 10,000 rpm for 1 minute at 25±2°C. The column was washed according to the standard protocol. For DNA elution, the spin column was placed in a fresh collection tube, 200 µL of elution buffer was added, incubated for 1 minute, and centrifuged at 10,000 rpm for 1 minute. The purified DNA was stored at –20°C until further molecular analysis.

Molecular characterisation of uropathogenic ESBL-producing isolates: All uropathogenic ESBL-producing *E. coli* isolates were subjected to molecular characterisation by amplification of selected ESBL-encoding genes, specifically CTX-M, SHV, and OXA-10/11. Detection was performed using the HiMedia Hi-PCR® ESBL Gene Probe PCR Kit, which enables identification of multiple ESBL genes in a single-tube multiplex PCR reaction. Prior to PCR amplification,

the reaction mixture was briefly centrifuged at 6,000 rpm for 10 seconds and then loaded into the BIO-RAD CFX96 Real-Time PCR System. Thermal cycling conditions were set according to the manufacturer's protocol provided with the kit.

Molecular detection of carbapenemase-encoding genes in *E. coli* isolates: All UPEC isolates confirmed phenotypically as carbapenemase producers were subjected to molecular characterisation through partial amplification of selected carbapenemase-encoding genes, including NDM, KPC, IMP, VIM, OXA-23, OXA-48, OXA-51 and OXA-58. Molecular detection was performed using the HiMedia Hi-PCR® Carbapenemase Gene (Multiplex) Probe PCR Kit, which allows sensitive and accurate identification of individual as well as co-existing carbapenemase genes in a single-tube multiplex reaction. Prior to PCR amplification, the reaction mixture was centrifuged at 6,000 rpm for 10 seconds and loaded into the BIO-RAD CFX96 Real-Time PCR System. Thermal cycling conditions included an initial denaturation at 95°C for 10 minutes, followed by 45 cycles of denaturation at 95°C for 5 seconds and a combined annealing/extension step at 60°C for 1 minute. Fluorescence signals were detected using the FAM, HEX, Texas Red, Cy5, and Cy5.5 channels. A final hold was maintained indefinitely at 4°C. Amplified products were analysed directly within the PCR system, and amplification curves were interpreted for final gene identification and reporting.

Extraction and Sample Preparation

Plant material of *Punica granatum* (peel) was collected from the herbal garden of Mahatma Gandhi Ayurved College, Wardha, and was identified and authenticated. The collected material was shade-dried and ground into a fine powder. Extraction was carried out using the Soxhlet method with 50% methanol as the solvent to isolate both water-soluble and alcohol-soluble bioactive phytochemicals. The resulting filtrate was concentrated using a rotary evaporator and stored at 4°C for further analysis.

Assessment of Antibacterial activity

The antibacterial activity of *Punica granatum* extract was evaluated using the standard Kirby-Bauer disc diffusion method, following general guidance from the Clinical and Laboratory Standards Institute (CLSI) [12]. ESBL- and carbapenemase-producing *Escherichia coli* isolates were adjusted to a 0.5 McFarland turbidity standard and uniformly inoculated onto Mueller-Hinton agar. Sterile filter paper discs (6 mm diameter) were impregnated with *P. granatum* extract at two concentrations (100% and 50%) and placed on the inoculated agar surface. Discs impregnated with 50% methanol served as the negative control, while commercially available gentamicin discs (10 µg) were used as the positive control, in accordance with CLSI guidelines. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured in millimetres. All experiments were performed in triplicate to ensure reproducibility and reliability, in accordance with previously reported phytochemical antibacterial studies [13].

Fourier Transform Infrared (FTIR) Analysis for Functional Group Identification

Functional group identification of bioactive phytochemicals present in the extract was carried out using Fourier Transform Infrared (FTIR) spectroscopy. Analysis was performed using a Bruker Alpha II FTIR spectrometer equipped with an Attenuated Total Reflectance (ATR) accessory. The sample was placed on the diamond ATR crystal surface and secured using the pressure knob after instrument calibration and baseline correction. Spectral data were acquired and processed, and absorption peaks were identified and interpreted based on their characteristic vibrational frequencies, with reference to the instrument's spectral library for functional group assignment.

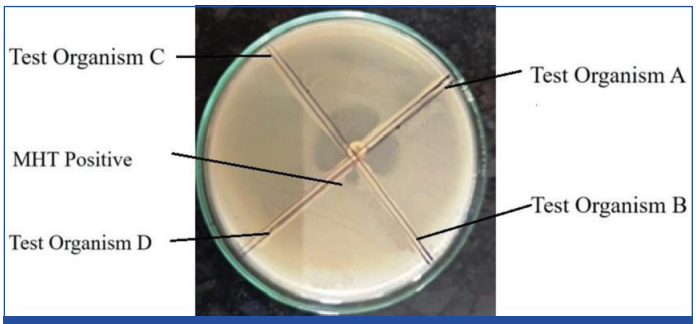
STATISTICAL ANALYSIS

All experiments were performed in triplicate, and results are expressed as mean±Standard Deviation (SD). The antibacterial activity of *Punica granatum* extract was statistically compared with that of the standard antibiotic gentamicin (10 µg/disc) using an unpaired Student's t-test. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 25.0 software.

RESULTS

Isolation, purification and identification of *E. coli*:

In the present study, the prevalence of MDR UPEC was 70.3%, with significant representation of ESBL-producing (82 isolates) and carbapenemase-producing (62 isolates) strains. Antibiotic susceptibility testing revealed prominent zones of inhibition. Phenotypic confirmation of ESBL- and carbapenemase-producing *E. coli* isolates using the Modified Double Disc Synergy Test (MDDST) and Modified Hodge Test (MHT) demonstrated reliable and consistent results [Table/Fig-1].



[Table/Fig-1]: Phenotypic confirmation by Modified Hodge Test (MHT) of Carbapenemase-producing *E. coli*.

Age wise Distribution of ESBL and Carbapenemase

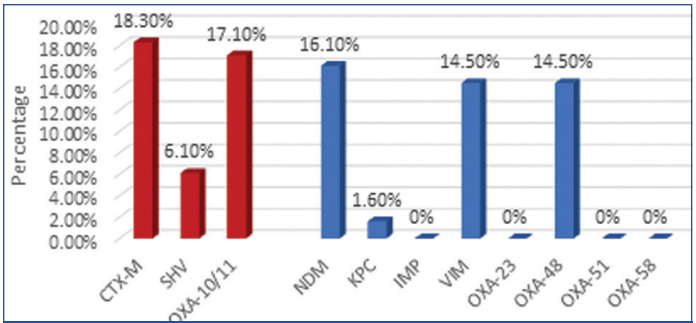
The highest incidence of ESBL production was observed in individuals aged 51-60 years, with 17 cases accounting for 20.7% of the total 82 ESBL-producing isolates, followed by the 41-50 years age group with 15 cases (18.3%) and the 61-70 age group with 14 cases (17.1%), 31-40 years showed 13 cases (15.8%) and 0-20 years and 71 and above showed 10 cases (12.1%). The lowest incidence was recorded in the 21-30 age group, with only 3 cases (3.7%).

A similar age-related trend was observed for carbapenemase production. Among the 62 carbapenemase-producing isolates, the highest prevalence was noted in the 51-60 years age group, accounting for 14 cases (22.6%), followed by the 31-40 years age group with 11 cases (17.7%), 41-50 years and 61-70 years showed 10 cases (16.13%), 71 years and above 7 cases (11.3%). The lowest numbers were observed in the 21-30 years and 0-20 years age groups, with 3 cases (4.8%) and 7 cases (11.3%), respectively.

Prevalence of ESBL and Carbapenemase Genes

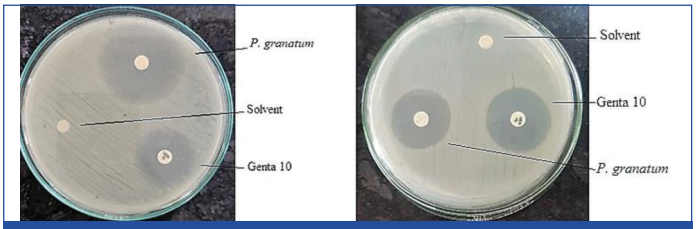
The prevalence of resistance genes was calculated as the proportion of phenotypically confirmed ESBL-producing (n=82) and carbapenemase-producing (n=62) *E. coli* isolates. Among the ESBL-producing isolates (n=82), the CTX-M gene was the most prevalent, detected in 15 isolates (18.3%). The OXA-10/11 gene was identified in 14 isolates (17.1%), while the SHV gene was comparatively less frequent, being detected in 5 isolates (6.1%). Among the carbapenemase-producing isolates (n=62), the NDM gene was detected in 10 isolates (16.1%), followed by the VIM and OXA-48 genes, each identified in 9 isolates (14.5%). The KPC gene was detected in only 1 isolate (1.6%). Carbapenemase genes such as IMP, OXA-23, OXA-51, and OXA-58 were not detected in any of the isolates [Table/Fig-2].

Antibacterial Activity of *Punica granatum* Extract



[Table/Fig-2]: Prevalence of ESBL and Carbapenemase Genes.

The methanolic extract of *Punica granatum* was evaluated against ESBL and carbapenemase-producing *E. coli* isolates using the disc diffusion method [Table/Fig-3,4].



[Table/Fig-3]: Antibacterial potential of *P. granatum* against Uropathogenic ESBL producing *E. coli* a) Sample with conc. 100%; b) Sample with conc. 50%. (Images from left to right)

[Table/Fig-4]: Antibacterial potential of *P. granatum* against Uropathogenic Carbapenemase producing *E. coli* a) Sample with conc. 100%; b) Sample with conc. 50%. (Images from left to right)

At 100% concentration, the extract produced a mean zone of inhibition of 21.18±1.4 mm against ESBL-producing strains, which was not statistically significantly different from the zone of 21.28±1.6 mm produced by the standard antibiotic Gentamicin (p-value=0.9812, unpaired Student's t-test). However, at 50% concentration, the mean zone of inhibition decreased to 16.92±1.1 mm, which was significantly lower than that of gentamicin (p-value <0.01), indicating a dose-dependent reduction in antibacterial activity.

Similarly, against carbapenemase-producing *E. coli*, the *P. granatum* extract at 100% concentration produced a mean zone of inhibition of 21.54±0.93 mm, which was comparable to that produced by Gentamicin (20.81±1.6 mm; p-value=0.32 58, unpaired Student's t-test). At 50% concentration, the inhibition zone declined to 17.9±0.6 mm, which was significantly lower than that of gentamicin (p-value <0.01) [Table/Fig-5].

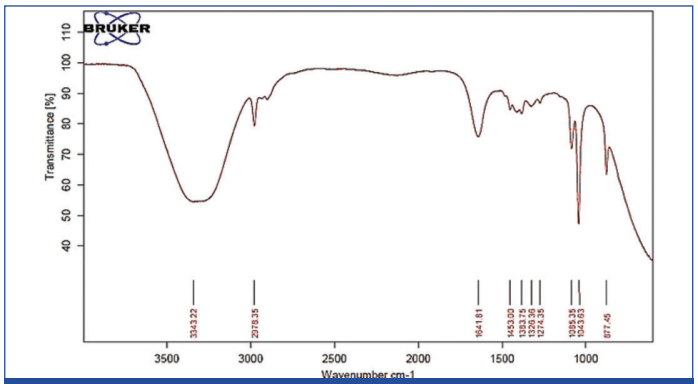
Statistical analysis using the unpaired (independent) Student's t-test revealed that, at 100% concentration, the antibacterial activity of *P. granatum* extract was comparable to that of gentamicin (p-value>0.05). In contrast, at 50% concentration, the reduction in inhibition zones was statistically significant (p-value <0.01), confirming decreased efficacy at lower concentrations.

FTIR Analysis of *Punica granatum* Extract

FTIR analysis revealed the presence of multiple functional groups in the *P. granatum* extract. A broad absorption peak at 3342.22 cm^{-1} indicated O-H stretching vibrations of alcohols and phenols. Peaks observed at 2978.35 cm^{-1} (C–H stretching), 1641.81 cm^{-1} (C=O or C=C stretching), and 1453 cm^{-1} (CH_2 bending) suggested the presence of aliphatic and aromatic structures. Additional peaks at 1383.75 cm^{-1}, 1274.35 cm^{-1}, 1085.35 cm^{-1}, 1043.63 cm^{-1}, and 877.45 cm^{-1} corresponded to methyl bending, C–O stretching, and out-of-plane C–H bending vibrations, respectively [Table/Fig-6].

Sample concentration (%)	Zone of inhibition of *P. granatum* extract (mm)	Zone of inhibition of standard gentamicin (mm)	t-value	p-value
ESBL producing UPEC				
100	21.18±1.4	21.28±1.6	-0.02	0.9812
50	16.92±1.1	21.28±1.6	-6.85	0.0001
Carbapenemase producing UPEC				
100	21.54±0.93	20.81±1.6	1.06	0.3258
50	17.9±0.6	20.81±1.6	-5.31	0.0019

[Table/Fig-5]: Antibacterial activity of *P. granatum* against genetically confirmed ESBL and Carbapenemase producing UPEC.
Test applied- Unpaired t-test



[Table/Fig-6]: FTIR spectrum of *P. granatum*.

DISCUSSION

In the present study, the prevalence of both ESBL and carbapenemase production increased with advancing age, particularly among individuals aged 41-70 years. These findings underscore the need for targeted, age-specific interventions and intensified surveillance in this demographic to effectively limit the spread of MDR UPEC. Molecular characterisation revealed a high prevalence of CTX-M and NDM genes, both of which are strongly associated with MDR. The absence of certain carbapenemase genes indicates variability in the distribution of resistance determinants, which may influence therapeutic decision-making and infection control strategies. This resistance gene distribution aligns with global antimicrobial resistance trends while also reflecting regional variability. Therefore, prioritising the detection and monitoring of predominant resistance genes—particularly CTX-M and NDM—is crucial for effective UTI management and the development of region-specific therapeutic strategies.

The methanolic extract of *Punica granatum* demonstrated potent antibacterial activity, particularly at 100% concentration, against both ESBL- and carbapenemase-producing UPEC strains. Although reduced activity was observed at lower concentrations, the extract exhibited substantial antibacterial potential, supporting further investigation into its therapeutic applications as a plant-based antimicrobial agent. The antimicrobial activity of the *Punica granatum* extract demonstrated a strong dose-dependent response, indicating its potential as a promising antibacterial agent comparable to conventional antibiotics. This activity is likely attributable to its bioactive compounds, which may disrupt bacterial cell walls and inhibit essential enzymatic pathways, particularly in ESBL- and carbapenemase-producing UPEC [14].

The ineffectiveness of 50% methanol against MDR *E. coli* in UTIs may be attributed to a combination of factors. *E. coli* possesses a complex outer membrane that is impermeable to certain solvents, such as methanol [15]. In addition, MDR *E. coli* strains often express efflux pumps capable of actively expelling toxic substances, such as methanol, from the bacterial cell [16]. Methanol may also exert solvent-related effects on the agar medium, potentially reducing its

antibacterial effectiveness [17]. Furthermore, MDR *E. coli* strains are known to form biofilms, which can protect bacteria from antimicrobial agents, including methanol [18]. These organisms may also harbour multiple resistance mechanisms, such as enzymatic inactivation and target modification, which further reduce susceptibility to methanol [19-21].

FTIR analysis confirmed the presence of key bioactive phytochemicals, including phenols, flavonoids, and tannins, which are well known for their antimicrobial properties. The FTIR spectrum revealed distinct absorption peaks corresponding to various functional groups within the sample. The broad absorption peak observed at approximately 3342.22 cm^{-1} indicated O–H stretching vibrations associated with alcohols, carboxylic acids, and phenols. The broad nature of this peak suggested extensive hydrogen bonding. Additionally, the peak at 2978.35 cm^{-1} was attributed to C–H stretching vibrations characteristic of CH_2 and CH_3 groups, indicating the presence of hydrocarbons or organic compounds with aliphatic chains. A distinct peak at 1641.81 cm^{-1} was attributed to C=O stretching vibrations, a characteristic feature of carbonyl-containing compounds such as ketones, esters, aldehydes, carboxylic acids, and amides. Alternatively, this peak may also correspond to C=C stretching vibrations commonly observed in alkenes and aromatic rings. The peak at 1453.00 cm^{-1} was associated with CH_2 bending vibrations typical of alkanes and may also indicate aromatic skeletal vibrations. In the subsequent spectral region, the peak at 1383.75 cm^{-1} corresponded to CH_3 bending vibrations, typically associated with methyl groups present in alkanes or branched hydrocarbons [22]. The presence of peaks at 1326.36 cm^{-1} and 1274.35 cm^{-1} suggested C–O stretching vibrations characteristic of esters, ethers, or carboxylic acids.

Similarly, the peaks at 1085.35 cm^{-1} and 1043.63 cm^{-1} also indicated C–O stretching vibrations, which may be attributed to alcohols, ethers, or polysaccharides, and could also reflect Si–O stretching if silicate compounds were present in the sample. Finally, the peak at 877.45 cm^{-1} likely corresponded to out-of-plane C–H bending vibrations, commonly observed in alkenes and aromatic compounds, and may also reflect carbohydrate-related functional groups [23].

Overall, the FTIR spectrum indicated the presence of hydroxyl, carbonyl, aliphatic, and ether or ester functional groups, suggesting a complex organic composition of polymeric or phytochemical origin and also support the presence of bioactive compounds such as flavonoids, phenols, terpenoids, and tannins. These phytochemicals are known to possess potent antimicrobial activity, particularly against MDR UPEC, a leading cause of UTIs [24,25].

Limitation(s)

One limitation of this study was that resistance genes were assessed individually, without specific analysis of the co-occurrence of multiple resistance genes within the same isolate. Future studies should employ advanced techniques such as multiplex PCR or whole-genome sequencing to identify the co-harbouring of ESBL and carbapenemase genes, as such combinations may contribute to increased resistance severity and therapeutic failure.

CONCLUSION(S)

This study provides compelling evidence of the strong in-vitro antibacterial activity of *Punica granatum* extract against MDR UPEC, including ESBL- and carbapenemase-producing strains. The exhibited efficacy highlights the potential of *P. granatum* as a sustainable and cost-effective phytotherapeutic option, particularly suitable for resource-limited settings. By integrating traditional knowledge with modern scientific validation, *P. granatum* shows promise as a complementary alternative to conventional antibiotics. However, further mechanistic studies and well-designed clinical trials are essential to translate these findings into effective therapeutic applications.

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Authors' contribution: NPK: Conducted the research experiments; PD: Supervision and project administration; NJB: Mentoring; SU: Supervision; SSG: Formal analysis; PSM: Data curation and manuscript drafting; AVH: Conceptualisation, manuscript writing, and editing.

REFERENCES

[1] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13(5):269-84. Doi: 10.1038/nrmicro3432.

[2] Mendes PM, Gomes Fontoura GM, Rodrigues LDS, Souza AS, Viana JPM, Fernandes Pereira AL et al. Therapeutic potential of *punica granatum* and isolated compounds: Evidence-based advances to treat bacterial infections. Int J Microbiol. 2023;2023:4026440. Published 2023 Dec 15. Doi:10.1155/2023/4026440.

[3] Mishra S, Modanwal S, Kumar P, Mishra A, Mishra N. Computational evaluation of *Punica granatum* leaf phytochemicals against multi-drug resistant *E. coli*: Molecular docking, ADMET, MD simulation, and DFT studies. Curr Comput Aided Drug Des. 2025. Available from: https://doi.org/10.2174/0115734099343126241105102839.

[4] Kiran S, Tariq A, Iqbal S, Naseem Z, Siddique W, Jabeen S, et al. Punicalagin, a pomegranate polyphenol sensitizes the activity of antibiotics against three MDR pathogens of the Enterobacteriaceae. BMC Complement Med Ther. 2024;24(1): 93. Available from: https://doi.org/10.1186/s12906-024-04376-7.

[5] Alghamdi MA, Al-Sarraj F, Alamshani WH, Alotibi I, Al-Zahrani M, Albiheyri R, et al. Antibacterial power of Pomegranate extracts against Beta-Lactamase producing *Escherichia coli*. Caryologia, 2024;77(1):83-99. Available from: https://doi.org/10.36253/caryologia-2356.

[6] Malik A, Bai S, Seasotiya L, Bharti P, Dalal S. Antibacterial and synergistic efficacy of *Punica granatum* ethyl acetate extract against multidrug resistant bacterial strains. Am J Adv Drug Deli. 2014;2(4):522-33. Available from: https://www.imedpub.com/articles/antibacterial-and-synergistic-efficacy-of-punica-granatum-ethyl-acetate-extract-against-multidrug-resistant-bacterialstrains.pdf.

[7] Alamshani WH, Al-Sarraj FA, Algamdi MA. The inhibitory effect of *Punica granatum* on *Escherichia coli* and *Klebsiella pneumonia* Extended spectrum β-lactamase strains. Novel Research in Microbiology Journal. 2023;7(1):1836-56. Available from: https://doi.org/10.21608/nrmj.2023.280475.

[8] Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG,et al; Infectious Diseases Society of America; European Society for Microbiology and Infectious Diseases. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011;52(5):e103-20. Doi: 10.1093/cid/ciq257. PMID: 21292654.

[9] Dehbanipour R, Rastaghi S, Sedighi M, Maleki N, Faghri J. High prevalence of multidrug-resistance uropathogenic *Escherichia coli* strains, Isfahan, Iran. J Nat Sci Biol Med. 2016;7(1):22-26.

[10] Raya GB, Dhoubhadel BG, Shrestha D, Raya S, Laghu U, Shah A, et al. Multidrug-resistant and extended-spectrum beta-lactamase-producing uropathogens in children in Bhaktapur, Nepal. Trop Med Health. 2020 Dec;48(1):65.

[11] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI supplement M100. Wayne, PA: CLSI; 2022.

[12] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2023.

[13] Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz J Microbiol. 2000;31(4):247-256. Doi: 10.1590/S1517-83822000000400003.

[14] Abdel-Aziz S, El-Esawi MA, Hazaa MM, Abdel-Aziz HY, Hassan MG. Antibacterial potential of pomegranate peel extracts on *Escherichia coli* isolated from Benha Hospital in Egypt. Benha J Appl Sci. 2021;6(3):61-64. Doi: 10.21608/bjas.2021.169813.

[15] Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. 2003;67(4):593-656. Doi: 10.1128/MMBR.67.4.593-656.2003.

[16] Piddock LJ. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clin Microbiol Rev. 2006;19(2):382-402.

[17] Hugo WB, Russell AD. Evaluation of the efficacy of antimicrobial agents against bacterial biofilms. J Appl Bacteriol. 1992;73(2):131-136.

[18] Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15(2):167-93.

[19] Wright GD. Bacterial resistance to antibiotics: Enzymatic degradation and modification. Adv Drug Deliv Rev. 2005;57(10):1451-70.

[20] Choi JG, Kang OH, Lee YS, Chae HS, Oh YC, Brice OO, et al. In vitro and in vivo antibacterial activity of *Punica granatum* peel ethanol extract against *Salmonella*. Evid Based Complement Alternat Med. 2011;2011:690518. Doi:10.1093/ecam/nep105.
[21] Silverstein RM, Webster FX, Kiemle DJ, Bryce DL. Spectrometric Identification of Organic Compounds. 8th ed. Wiley; 2014.
[22] Smith BC. Infrared Spectral Interpretation: A Systematic Approach. CRC Press; 2011.
[23] Pavia DL, Lampman GM, Kriz GS, Vyvyan JR. Introduction to Spectroscopy (5th ed.). Cengage Learning; 2014.
[24] Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-82. Available from: https://doi.org/10.1128/CMR.12.4.564.
[25] Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343-56. Available from: https://doi.org/10.1016/j.ijantimicag.2005.09.002.

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