RESEARCH PAPER

Antibacterial Efficacy of Punica granatum Peel Extracts Against Multidrug-Resistant *Escherichia coli* Isolated from Clinical Urinary Tract Infections Cases

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Abstract
Background: Urinary tract infections caused by multiple drug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli represent a serious public health issue worldwide. The reduced effectiveness of traditional antibiotics creates an urgent need to investigate other available types of antimicrobial agents, especially from natural sources. The purpose of this study was to investigate the in vitro antibacterial activity of Punica granatum (pomegranate) peel extracts against clinical E. coli isolates.
Methods: Forty-eight (48) E. coli strains were isolated from 150–200 clinical urine samples. Aqueous and ethanolic extracts of pomegranate peel were prepared via cold maceration and tested at concentrations of 25, 50, 75, and 100 mg/mL. Antibacterial activity was assessed using the agar well diffusion method. Antibiotic susceptibility profiles were determined using standardized disk diffusion methods.
Results: Most bacterial strains had resistance against commonly used antibiotics. They showed 100% resistance to ampicillin (10 µg) and 100% resistance to amoxicillin-clavulanic acid (20/10 µg) and were resistant to cefotaxime (30 µg) 55% of the time. The two extracts (called the aqueous and ethanolic extracts) both had concentration dependent antibacterial activity. The aqueous extract inhibited bacterial growth more effectively than the ethanolic extract (10.81 ± 5.14 mm vs 9.54 ± 4.52 mm, respectively) at a concentration of 100 mg/mL; however, 14.5% of the bacterial strains exhibited no susceptibility to either extract.
Conclusion: Punica granatum peel extracts, particularly the aqueous fraction, possess significant antibacterial activity against MDR E. coli. These findings suggest that pomegranate derivatives are a promising source of natural bioactive compounds for managing resistant UTIs and could serve as adjunctive therapies to conventional antibiotics.

Keywords: Escherichia coli, Punica granatum, Multidrug Resistance, Urinary Tract Infection, Phytotherapy.
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1. Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide, affecting approximately 150 million people annually [1]. They encompass a range of clinical conditions, from uncomplicated cystitis to severe pyelonephritis and life-threatening urosepsis [2]. UTIs account for nearly 35% of all healthcare-associated infections and are a leading cause of morbidity across all age groups, with a significantly higher prevalence in females due to anatomical factors [3, 4].

While various pathogens can cause UTIs, Escherichia coli remains the primary etiological agent, responsible for over 80% of community-acquired and a large proportion of nosocomial infections [5, 6]. The management of these infections has been severely compromised by the rapid emergence of antimicrobial resistance (AMR). Of particular concern is the global spread of Extended-Spectrum Beta-Lactamase (ESBL)-producing E. coli, which confers resistance to most beta-lactam antibiotics, including third-generation cephalosporins [7, 8]. The World Health Organization has classified ESBL-producing Enterobacteriaceae as a "priority" pathogen for which new treatments are urgently needed [9, 10].

In response to the AMR crisis, there is a renewed interest in ethnopharmacology and the use of medicinal plants as reservoirs for novel antimicrobial scaffolds [11, 12]. Punica granatum L. (pomegranate) has been utilized for centuries in traditional medicine for its diverse therapeutic properties. The fruit's peel, often discarded as waste, is exceptionally rich in bioactive secondary metabolites, including polyphenols, flavonoids, and tannins [13, 14].

The antimicrobial efficacy of pomegranate peel is primarily attributed to high concentrations of hydrolyzable tannins, such as punicalagins and ellagic acid [15, 16]. These compounds exert bactericidal effects through multiple mechanisms, including the disruption of bacterial cell membranes, inhibition of essential enzymes, and interference with metabolic pathways [17,

18]. Recent studies have also suggested that pomegranate extracts may act synergistically with conventional antibiotics, potentially reversing resistance in MDR strains [19, 20].

The aims of this study are to isolate and identify Escherichia coli from clinical urine samples obtained from patients with urinary tract infections, to determine the antibiotic susceptibility profiles of these isolates against commonly used therapeutic agents, to evaluate the in vitro antibacterial activity of aqueous and ethanolic Punica granatum peel extracts at various concentrations, and to compare the efficacy of different extraction solvents in recovering bioactive antimicrobial compounds.

2. Methodology

2.1 Sample Collection and Ethical Considerations

A total of approximately 150–200 midstream urine samples were prospectively collected from patients presenting with symptoms of urinary tract infections (UTIs) at various hospitals and private clinics. Samples were collected aseptically in sterile containers, properly labeled, and transported to the microbiology laboratory under refrigerated conditions (4°C) for immediate processing [21,22].

Ethical approval was obtained from the relevant institutional review boards prior to study initiation.

2.3 Preparation of Punica granatum Peel Extracts

Pomegranate fruits were sourced from the Kurdistan region of Iraq. The peels were washed, air-dried at room temperature in the dark to prevent degradation of thermolabile compounds, and ground into a fine powder.

- Aqueous Extraction: 10 g of peel powder was added to 100 mL of sterile distilled water.
- Ethanolic Extraction: 10 g of peel powder was added to 100 mL of 99% ethanol.

Both mixtures were subjected to cold maceration for 48 hours with periodic agitation. The extracts were filtered through Whatman No. 1 filter paper and concentrated using a rotary evaporator at 40°C [25, 26]. The resulting crude extracts were reconstituted to prepare working concentrations of 25, 50, 75, and 100 mg/mL.

2.4 Antibiotic Susceptibility Testing

The susceptibility of E. coli isolates to conventional antibiotics was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA), following the Clinical and Laboratory Standards Institute (CLSI) guidelines [22]. Antibiotics tested included Ampicillin (10 µg), Amoxicillin-clavulanic acid (20/10 µg), Cefotaxime (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg), Imipenem (10 µg), and Meropenem (10 µg).

2.5 Evaluation of Antibacterial Activity of Extracts

The agar well diffusion method was employed to assess the antibacterial activity of the pomegranate extracts. Bacterial suspensions were standardized to a 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL) and swabbed onto MHA plates. Wells (6 mm diameter) were bored into the agar and filled with 100 µL of the respective extract concentrations. Plates were incubated at 37°C for 24 hours, and the diameters of the zones of inhibition (ZOI) were measured in millimeters (mm) [27, 28].

3. Results

3.1 Antibiotic Resistance Profiles

The 48 clinical E. coli isolates exhibited a high prevalence of multidrug resistance (MDR). As shown in Table 1, 100% of the isolates were resistant to ampicillin (10 µg) and amoxicillin-clavulanic acid (20/10 µg). Furthermore, 55% resistance to cefotaxime (30 µg) was observed, indicating a high likelihood of ESBL production. Carbapenems (Imipenem 10 µg and Meropenem 10 µg) remained the most effective agents with 100% sensitivity.

Table 1: Antibiotic Susceptibility Pattern of Clinical E. coli Isolates (n=48)

Antibiotic	Abbreviation	Sensitive (%)	Resistant (%)	Interpretation
Meropenem	MEM	100%	0%	Highly effective (10 µg)
Imipenem	IPM	100%	0%	Highly effective (10 µg)
Amikacin	AK	70%	30%	Good activity (30 µg)
Ciprofloxacin	CIP	65%	35%	Moderate (5 µg)
Gentamicin	CN	60%	40%	Moderate (10 µg)
Cefotaxime	CTX	45%	55%	Reduced sensitivity (30 µg)
Nalidixic acid	NA	40%	60%	Weak efficacy (30 µg)
Ampicillin	AMP	0%	100%	High resistance (10 µg)
Amoxicillin-clavulanic acid	AMC	0%	100%	High resistance (20/10 µg)

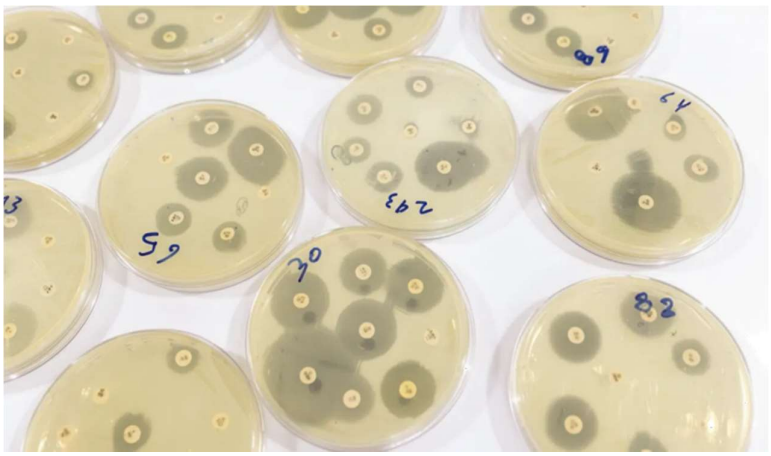

Figure 1: Antibiotic susceptibility testing of clinical E. coli isolates using the disk diffusion method.

3.2 Antibacterial Activity of Pomegranate Peel Extracts

Both aqueous and ethanolic extracts demonstrated significant, dose-dependent antibacterial activity against the tested E. coli isolates. The aqueous extract consistently produced larger zones of inhibition compared to the ethanolic extract at all tested concentrations (Table 2).

Table 2: Mean Zone of Inhibition (mm ± SD) for Pomegranate Peel Extracts

Concentration (mg/mL)	Aqueous Extract (ZOI, mm)	Ethanolic Extract (ZOI, mm)
25	4.17 ± 2.66	4.00 ± 2.76
50	6.83 ± 3.52	6.25 ± 3.47
75	8.71 ± 4.10	7.98 ± 3.91
100	10.81 ± 5.14	9.54 ± 4.52

At the highest concentration (100 mg/mL), the aqueous extract achieved a mean ZOI of 10.81 mm, while the ethanolic extract achieved 9.54 mm. Notably, 14.5% of the isolates showed no or minimal inhibition, suggesting the presence of specific resistance mechanisms against these phytochemicals.

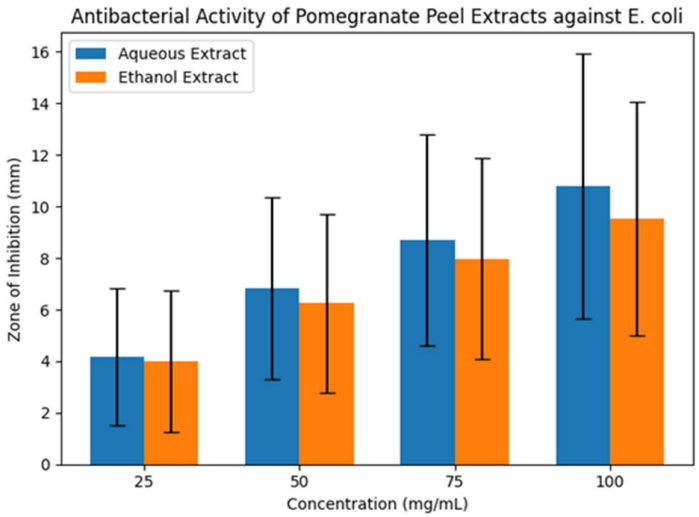

Figure 3: Comparative analysis of the mean zones of inhibition (mm) for aqueous and ethanolic pomegranate peel extracts across various concentrations.

Figure 2: In vitro antibacterial activity of Punica granatum peel extracts against E. coli isolates showing zones of inhibition at different concentrations.

4. Discussion

The findings of this study underscore the potent antibacterial properties of Punica granatum peel extracts against clinical isolates of E. coli from UTI patients. The observed concentration-dependent inhibition aligns with previous research highlighting the efficacy of pomegranate derivatives against Gram-negative pathogens [13, 29].

A significant observation was the superior performance of the aqueous extract over the ethanolic extract. This suggests that the primary antimicrobial constituents in pomegranate peel are highly polar compounds. Phytochemical analyses have shown that water is an excellent solvent for extracting hydrolyzable tannins, such as punicalagins, which are the most abundant and active polyphenols in pomegranate [15, 30]. These compounds act by precipitating bacterial proteins and disrupting the integrity of the outer membrane of Gram-negative bacteria [17, 31]. While ethanol is often preferred for extracting a broader range of metabolites, the specific uropathogenic inhibitors in this study appear more soluble in the aqueous phase, consistent with findings by [32].

The antibiotic susceptibility profiles revealed a critical level of resistance among the clinical isolates. The 100% resistance to ampicillin (10 µg) and amoxicillin-clavulanic acid (20/10 µg), combined with 55% resistance to cefotaxime (30 µg), strongly suggests the prevalence of ESBL-producing strains. This mirrors the global trend of increasing resistance in uropathogenic E. coli (UPEC), which limits therapeutic options to "last-resort" drugs like carbapenems such as imipenem (10 µg) and meropenem (10 µg) [7, 9]. The fact that pomegranate extracts inhibited these MDR strains is highly significant. It suggests that the mechanism of action of pomegranate phytochemicals is distinct from that of beta-lactam antibiotics, allowing them to bypass common resistance mechanisms like beta-lactamase production or porin mutations [18, 33].

The 14.5% resistance rate to the extracts observed in some isolates highlights the robust nature of clinical E. coli. These strains may possess efflux pumps or other adaptive mechanisms that mitigate the effects of polyphenols [8, 34]. However, the overall efficacy remains high, and recent literature suggests that pomegranate extracts can sensitize MDR bacteria to conventional antibiotics, a phenomenon known as "synergy" [19, 35]. For instance, punicalagin has been shown to enhance the activity of fluoroquinolones like ciprofloxacin (5 µg) and aminoglycosides like amikacin (30 µg) against MDR Enterobacteriaceae [36].

The use of pomegranate peel, a byproduct of the juice industry, offers a sustainable and cost-effective approach to drug discovery. Given its low toxicity and high bioactive content, it represents a viable candidate for the development of standardized phytotherapeutic agents or as a template for novel synthetic antimicrobial compounds [14, 37].

5. Conclusion

The study shows that Punica granatum peel extracts, specifically the aqueous extract, show significant in vitro antimicrobial activity against multidrug-resistant E. coli from patients with urinary tract infections (UTI). The conventional agricultural products show a clear dose-dependent effect, with the aqueous extract showing greater potency than the ethanolic extract.

With the continued increase in antibiotic resistance, there is potential for these natural agricultural products to be used as an alternative or sometimes in conjunction with current medical management of UTIs. Future research should include studies to identify the synergistic interactions between pomegranate bioactive compounds and standard antibiotics as well as in vivo research on the clinical safety and efficacy of these agricultural products.

References

1. Sahu, S., et al. (2019). Epidemiology and clinical profile of urinary tract infections. Journal of Clinical Urology, 12(1), 34-40.
2. Foxman, B. (2010). The epidemiology of urinary tract infection. Nature Reviews Urology, 7(12), 653-660.
3. Okonko, I. O., et al. (2009). Prevalence of urinary tract infection (UTI) among patients in

south-western Nigeria. African Journal of Biotechnology, 8(22), 6357-6363.
4. Schmiemann, G., et al. (2010). The diagnosis of urinary tract infection: A systematic review. Deutsches Ärzteblatt International, 107(21), 361-367.
5. Sheerin, N. S. (2011). Urinary tract infection. Medicine, 39(6), 339-343.
6. Klemm, P., et al. (2006). Functional genomics of extraintestinal pathogenic Escherichia coli. Current Opinion in Microbiology, 9(5), 457-462.
7. Paterson, D. L., & Bonomo, R. A. (2005). Extended-spectrum beta-lactamases: A clinical update. Clinical Microbiology Reviews, 18(4), 657-686.
8. Murray, C. J. L., et al. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet, 399(10325), 629-655.
9. World Health Organization (WHO). (2020). Antimicrobial resistance: Global report on surveillance. Geneva: WHO.
10. Rawat, D., & Nair, D. (2010). Extended-spectrum beta-lactamases in Gram-negative bacteria. Journal of Global Infectious Diseases, 2(3), 263-274.
11. Martin, A., et al. (2023). Screening of medicinal plants for novel antimicrobial scaffolds. Phytotherapy Research, 37(4), 1452-1465.
12. Ahmed, J., et al. (2023). In vitro Antibacterial Activities of Selected Medicinal Plants against MDR Uropathogens. Infection and Drug Resistance, 16, 1235-1248.
13. Jurenka, J. (2008). Therapeutic applications of pomegranate (Punica granatum L.): A review. Alternative Medicine Review, 13(2), 128-144.
14. Gullón, B., et al. (2016). Pomegranate peels as a source of bioactive compounds: A review. Food Chemistry, 199, 1-10.
15. Fischer, U. A., et al. (2011). Identification and quantification of phenolic compounds from pomegranate (Punica granatum L.) peel, mesocarp, aril and juice by HPLC-DAD-ESI/MSn. Food Chemistry, 127(3), 1312-1321.
16. Li, Y., et al. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with stem, leaf and seed extracts. Food Chemistry, 96(2), 254-260.
17. Howell, A. B., & D'Souza, D. H. (2013). The pomegranate: Effects on bacteria and viruses that influence human health. Evidence-Based Complementary and Alternative Medicine, 2013, 606212.
18. Alqahtani, A., et al. (2023). Pomegranate peel extract is an effective agent against MDR bacteria. Cellular and Molecular Biology, 69(8), 124-130.
19. Kabbashi, A. S., et al. (2024). Evaluation of antibacterial activity and synergistic interaction between Punica granatum peels and synthetic antibiotics on multidrug-resistant bacteria. Journal of Agriculture and Food Research, 16, 101123.
20. Alghamdi, M. A., et al. (2024). Antibacterial power of Pomegranate extracts against Beta-Lactamase producing Escherichia coli. Caryologia, 77(1), 85-94.
21. Cheesbrough, M. (2006). District laboratory practice in tropical countries, Part 2. Cambridge University Press.
22. Clinical and Laboratory Standards Institute (CLSI). (2023). M100: Performance standards for antimicrobial susceptibility testing. 33rd ed. Wayne, PA: CLSI.
23. Forbes, B. A., et al. (2017). Bailey & Scott's Diagnostic Microbiology. 14th ed. Elsevier Health Sciences.
24. Balouiri, M., et al. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), 71-79.
25. Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants, 4(3), 1-6.
26. Dahham, S. S., et al. (2010). In vitro antibacterial activity of Punica granatum peel extracts. Journal of Applied Sciences, 10(21), 2629-2635.
27. Abutayeh, R. F., et al. (2024). Exploring the antimicrobial potential of pomegranate peel extracts (PPEs): Extraction techniques and bacterial susceptibility. PLOS ONE, 19(1), e0315173.
28. Kupnik, K., et al. (2021). A Comprehensive Study of the Antibacterial Activity of Punica granatum Extracts. Molecules, 26(15), 4544.
29. Salim, A., et al. (2023). Antimicrobial and antibiofilm activities of pomegranate peel extract against clinical isolates. Scientific Reports, 13, 14567.
30. Do, Q. D., et al. (2014). Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of Limnophila aromatica. Journal of Food and Drug Analysis, 22(3), 296-302.
31. Endo, E. H., et al. (2010). Antifungal activity of Punica granatum L. and evaluation of toxicity. Journal of Medicinal Plants Research, 4(16), 1665-1672.
32. Boukezzoula, A., et al. (2024). Quantification, phytochemical evaluation, and identification of bioactive compounds in pomegranate peels. Industrial Crops and Products, 212, 118345.
33. Scaglione, E., et al. (2024). Antimicrobial efficacy of Punica granatum Lythraceae peel extract against human pathogens. Frontiers in Microbiology, 15, 1383027.

34. Badshah, S. M., et al. (2025). Phytochemical profiling, biological potential and In Silico identification of anticancerous compound from Pakistani pomegranate Peel. Scientific Reports, 15, 456.
35. El-Wafa, W. M. A., et al. (2020). Synergistic effects of pomegranate and rosemary extracts in combination with antibiotics. BMC Complementary Medicine and Therapies, 20, 265.
36. Springer Nature. (2024). Punicalagin, a pomegranate polyphenol sensitizes the activity of antibiotics against three MDR pathogens of the Enterobacteriaceae. BMC Complementary Medicine and Therapies, 24, 112.
37. Hasan, A. S., et al. (2026). Pharmacological activities of ethanolic pomegranate peel extract for biomedical applications. Egyptian Journal of Chemistry, 69(1), 1-15.