

Original Research

Comparison of Antimicrobial Activity of Punica granatum Seed Extract with Antibiotics against Escherichia coli and Streptococcus mutans

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ABSTRACT:

The emergence of antibiotic-resistant bacterial strains has intensified the search for alternative natural antimicrobials. This study evaluated the antibacterial activity of Punica granatum (pomegranate) seed extract, tetracycline fibers, and amoxicillin against Escherichia coli (MTCC 23790) and Streptococcus mutans (MTCC 24383). Experiments were conducted under sterile conditions at the PAU Microbiology Laboratory, Ludhiana. Antimicrobial efficacy was assessed using the agar well diffusion method, with inhibition zones measured after 24 h (E.coli) and 48 h (S.mutans). Tetracycline and amoxicillin produced the largest inhibition zones, whereas P.granatum seed extract demonstrated significant antibacterial activity, particularly against E. coli. These findings highlight the potential of P.granatum seed extract as a natural antimicrobial agent and support its further investigation as an adjunct or alternative to conventional antibiotics.

Keywords: Punica granatum, antimicrobial activity, Escherichia coli, Streptococcus mutans, tetracycline, amoxicillin, MTCC.

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INTRODUCTION

Background

The development of antibiotics revolutionized infection control; however, indiscriminate use has led to multidrug-resistant bacteria, threatening global health (WHO, 2022). The search for novel antimicrobial agents includes natural compounds with minimal side effects.

Medicinal Significance of Punicagranatum

Punica granatum L. (pomegranate) is widely recognized in traditional medicine for its therapeutic properties. Its seeds contain polyphenols, flavonoids, tannins, and alkaloids, which exhibit antimicrobial, antioxidant, and anti-inflammatory effects (Baian AL Dobayan, 2021).

Seed-derived extracts have been shown to inhibit the growth of Gram-positive and Gram-negative bacteria through membrane disruption and enzyme

interference (Prashanth et al., 2001; Reddy et al., 2007).

Rationale of the Study

Given increasing antibiotic resistance, exploring plant-based antimicrobials as adjuncts or alternatives to conventional antibiotics is imperative. Comparing P. granatum seed extract with tetracycline and amoxicillin can provide insights into its relative efficacy against both Gram-positive and Gram-negative bacteria.

Objectives

This study aimed to:

1. Evaluate the antimicrobial activity of P.granatum seed extract against E. coli and S.mutans.
2. Compare its inhibitory effect with tetracycline fibers and amoxicillin. Gram-positive and Gram-negative bacterial

strains.

MATERIALS

Materials and Methods

- Plant material: Punica granatum seeds were collected, washed, and crushed to obtain juice.
- Filtration: The juice was filtered through a sterile syringe filter (0.22 micrometer pore size) to remove particulate matter.
- Antibiotics: Tetracycline fibers (2mg) and amoxicillin were used as positive controls.
- Bacterial strains: E.coli(MTCC 23790) and S.mutans (MTCC 24383), obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh.
- Culture media: Mueller-Hinton agar (S.mutans) and EMB Broth (E.coli).

Inoculum Preparation

Both the bacteria were grown overnight in nutrient broth at 37 °C. Turbidity was adjusted to 0.5 McFarland standard ($\sim 1 \times 10^8$ CFU/mL).

Antimicrobial Assay

The agar well diffusion method was employed:

1. Inoculation: 10 μ L of bacterial culture was uniformly spread on agar plates.
2. Well preparation: Wells of 4 mm diameter were aseptically bored.
3. Addition of agents: Wells were filled with P. granatum seed extract (0.5 mL), tetracycline fibers (0.018 g), or amoxicillin (100 mg).
4. Incubation: Plates were incubated at 37 °C for 48 h (E.coli) and 48 h (S. mutans).
5. Measurement: Zones of inhibition were measured in centimeters using a digital caliper.

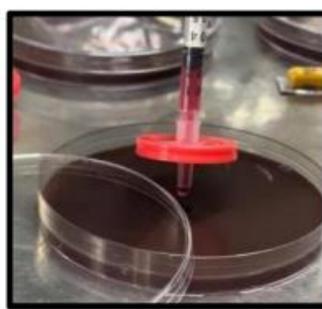
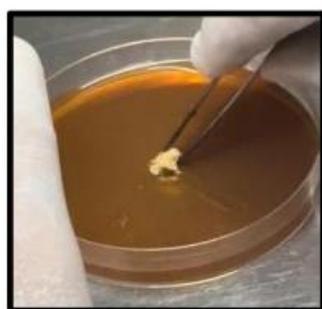


Figure: Agar well diffusion assay. (a) Laminar air flow. Wells (4 mm diameter) were filled with the respective agents, (b) tetracycline fibers, (c) Punica granatum seed extract, (d) amoxicillin powder and plates were incubated at 37 °C for 48 h. Zones of inhibition were measured in centimeters using a digital caliper.

Statistical Analysis

All assays were performed in triplicate. Results are presented as mean \pm standard deviation. Statistical significance was evaluated using one-way ANOVA with Tukey's post hoc test ($p < 0.05$).

RESULTS**Antimicrobial Activity**

Test Agent	<i>E. coli</i> (cm)	<i>S. mutans</i> (cm)
Punica granatum seed extract	2.4 ± 0.15	1.3 ± 0.10
Tetracycline fibers	4.0 ± 0.20	3.0 ± 0.20
Amoxicillin	5.65 ± 0.30	5.0 ± 0.25

Tetracycline and amoxicillin produced the largest zones, while P.granatum seed extract demonstrated moderate but significant antimicrobial activity. *E. coli* was more susceptible to the extract than *S.mutans*

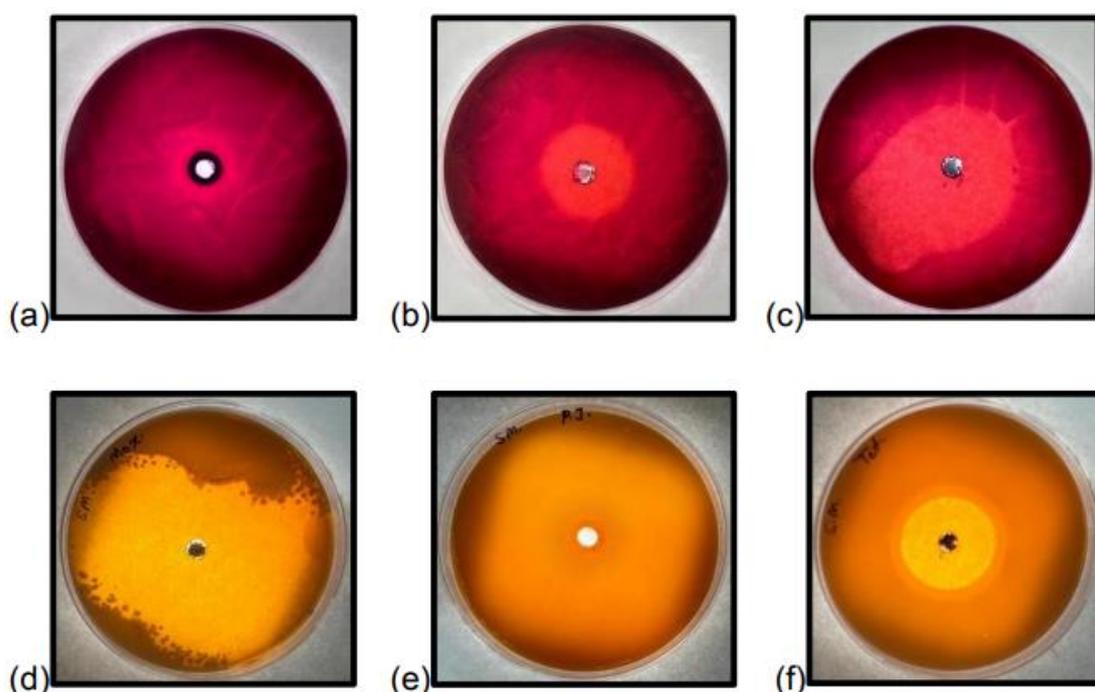


Figure: Zones of inhibition exhibited by different test agents against (a–c) *E. coli* and (d–f) *S. mutans*. (a, e) Punica granatum seed extract; (b, f) tetracycline fibers; (c, d) amoxicillin. Tetracycline and amoxicillin showed the largest inhibition zones, whereas P. granatum seed extract exhibited moderate antimicrobial activity, with *E. coli* being more susceptible than *S. mutans*.

Observations

The ± values reflect minor experimental variations among triplicates, which is common due to differences in bacterial growth density, agar consistency, and diffusion of agents.

DISCUSSION**Interpretation of Results**

Punica granatum seed extract demonstrated measurable antibacterial activity against both Gram-positive (*S.mutans*) and Gram-negative (*E.coli*) bacteria, although the inhibition zones were smaller than those produced by tetracycline and amoxicillin. This confirms that the extract contains active constituents capable of inhibiting bacterial growth under in vitro conditions.

Interestingly, *E.coli* exhibited greater susceptibility (2.4 ± 0.15 cm) compared to *S. mutans* (1.3 ± 0.10 cm). This difference can be attributed to the variations in bacterial cell wall architecture. Gram-negative bacteria like *E.coli* possess an outer membrane containing lipopolysaccharides that allow diffusion of small hydrophobic molecules, including polyphenols, while the thicker peptidoglycan layer of Gram-positive bacteria such as *S.mutans* may restrict diffusion of certain phytochemicals (Reddy et al., 2007).

Bioactive constituents in *P.granatum* seeds such as punicalagin, ellagic acid, and catechins are known for their antimicrobial and antioxidant effects (Baian, 2021). These compounds have been shown to inhibit a range of pathogens by disrupting membrane integrity

and impairing enzyme function (Prashanth et al., 2001). The findings of this study are therefore consistent with earlier research reporting that pomegranate extracts exhibit inhibitory effects against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E. coli* (Reddy et al., 2007; Baian, 2021).

Mechanisms of Action

The antimicrobial effects of *P. granatum* seed extract are likely due to the synergistic action of polyphenols, flavonoids, and tannins. These compounds can cause membrane disruption, enzyme inhibition, and leakage of intracellular contents, ultimately leading to cell death (Reddy et al., 2007). Additionally, polyphenols can generate reactive oxygen species (ROS), leading to oxidative damage of bacterial proteins and lipids (Baian, 2021).

Unlike single-target antibiotics such as tetracycline, which binds to the 30S ribosomal subunit, or amoxicillin, which inhibits bacterial cell wall synthesis via β -lactam activity, *P. granatum* acts through multiple mechanisms (Carranza, 2012). This multitarget approach may reduce the likelihood of resistance development, making plant-based antimicrobials valuable alternatives or adjuncts to conventional antibiotics.

Recent studies also suggest that pomegranate extracts may interfere with quorum sensing—the bacterial communication system responsible for virulence and biofilm formation (Salgado et al., 2006). This property is particularly relevant to *S. mutans*, which forms resilient biofilms in dental plaque, contributing to caries development.

Comparison with Literature

The antimicrobial activity observed in this study agrees with previous reports on *P. granatum* extracts. Prashanth et al. (2001) reported inhibition zones ranging from 1.5 to 2.8 cm for *E. coli* and *S. aureus*, which are comparable to the present findings. Similarly, Reddy et al. (2007) demonstrated significant bactericidal effects of tannin-rich fractions from *P. granatum*, highlighting the role of ellagitannins in disrupting bacterial cell membranes.

Baian (2021) found that ethanolic pomegranate seed extracts were particularly effective against *E. coli* and *Klebsiella pneumoniae*, supporting the current observation that Gram-negative bacteria may be more susceptible to pomegranate-derived compounds. In the dental field, Salgado et al. (2006) showed that natural plant extracts, including pomegranate, were effective against oral pathogens, suggesting possible use in periodontal therapy and mouth rinses. These collective findings reinforce the potential of *P. granatum* as a broad-spectrum antimicrobial.

Broader Implications

The increasing prevalence of antibiotic resistance underscores the urgent need for alternative therapeutic options (WHO, 2022). Plant-derived antimicrobials

such as *P. granatum* provide promising candidates due to their safety, availability, and ability to act through multiple pathways. The demonstrated antimicrobial activity against both Gram-positive and Gram-negative bacteria supports potential applications in topical antiseptics, oral care formulations, and food preservation (Baian, 2021).

Moreover, combining *P. granatum* extract with conventional antibiotics may result in synergistic effects, enhancing antibacterial efficacy while reducing required dosages and minimizing resistance (Salgado et al., 2006). Such strategies align with the current global movement toward integrating natural and synthetic antimicrobials in infection management.

Study Limitations

- In vitro conditions may not fully replicate in vivo effects.
- Concentration-dependent activity of the extract was not fully explored.
- Only two bacterial strains were tested.

Future Recommendations

- Isolation and characterization of active compounds from seeds.
- Evaluation of synergistic effects with conventional antibiotics.
- In vivo testing to assess pharmacological efficacy and safety.

CONCLUSION

Punica granatum seed extract exhibits significant antimicrobial activity against *E. coli* (MTCC 23790) and *S. mutans* (MTCC 24383), though less potent than tetracycline and amoxicillin. Its broad-spectrum activity, particularly against *E. coli*, suggests its potential as a natural antimicrobial agent. Further research is warranted to explore its clinical applications as an adjunct or alternative to antibiotics.

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