Investigating the Antimicrobial Properties of *Punica granatum*

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Abstract

Each year, hundreds of millions of antibiotics are administered to people and to animals. The overuse and misuse of antibiotics has led to a growing public health crisis—antibiotic resistance. This crisis calls for new antimicrobial compounds to combat resistant pathogens. Plants used by traditional healers may provide these new antimicrobics. *Punica granatum* (pomegranate) has been used to treat skin wounds in Middle Eastern and Asian medicine. Our aim is to explore the antimicrobial properties of *P. granatum* seeds against bacteria and fungi. Acetonic, isopropanolic, and aqueous extracts of pomegranate seeds (250 mg/mL) were screened against methicillin-resistant *Staphylococcus aureus* (MRSA), *Mycobacterium phlei, Enterococcus faecalis,* and *Streptococcus mutans* (gram-positive) bacteria; *Escherichia coli* (gram-negative) bacteria; and *Aspergillus niger* (fungus) using agar diffusion assays. The gram-positive bacteria, including MRSA, were inhibited by isopropanolic extracts. Using serial dilutions, the minimal inhibitory and bactericidal concentration against *S. aureus* is 125 mg/mL. We tested the effect of temperature (ranging from 5-56°C) on the extracts' antimicrobial properties. The greatest inhibition was seen at 25°C. Chromatography was used to isolate the antimicrobial component of the extract. These findings and further studies may provide an alternative antimicrobial against antibiotic-resistant gram-positive bacteria.

Hypothesis

Punica granatum inhibits the growth of *Staphylococcus aureus* (gram-positive) bacteria and *Escherichia coli* (gram-negative) bacteria and *Aspergillus niger* fungus.

Background

- In recent years, the need for new antimicrobics has been increasing due to antibiotic resistance (4).
- Plants are a potential source of new antimicrobics (4).
- *Punica granatum* (**Figure 1**) has a long history of use in Greek, Egyptian and Chinese traditional healing (1).
- Preliminary studies suggest that *P. granatum* has potential antimicrobial property against *Staphylococcus aureus* bacteria and fungi (2, 3).
- The purpose of our work is to determine whether *P. granatum* inhibits gram-positive bacteria, gram-negative bacteria, and fungi.

Figure 1. *Punica granatum*, pomegranate, is in the family Lythraceae. It is native to regions in Asia, from the Middle East to the Himalayas, and is also widely cultivated now in parts of Southwest America, California, Mexico, Arizona and Africa. Plant photograph: USDA.

Materials & Methods

1. Commercial dried pomegranate seeds were purchased from local markets.
2. 250 mg/mL extracts were made by grinding 1 g pomegranate seeds in 4 mL acetone, 70% isopropyl, or water with a mortar and pestle.
3. Well diffusion assays with 40 µL of extract per well were used to determine antimicrobial activity. Antibiotic disks (Hardy Diagnostics) were used as positive controls (**Table 1**). 40 µL of the solvent used to make the extracts was used as negative controls.
4. The minimal inhibitory and bactericidal concentrations against *S. aureus* were determined by inoculating serial dilutions (500-0.000119 mg/mL) of the isopropanolic extract in nutrient broth in cell well plates.
5. 250 mg/mL of aqueous and isopropanolic extracts were incubated for 30 min at 5°C, 25°C, and 56°C. Well diffusion assays were then used to determine the effect of temperature on pomegranate's antibacterial activity against *S.aureus*.
6. Antimicrobial compounds were separated by paper chromatography in NaOH-isopropyl alcohol. Disk diffusion assays on the chromatogram were used to locate antimicrobial compounds.

Table 1. Organisms used in disk-diffusion assays.

Organism	Source	Positive control
Staphylococcus aureus	ATCC 27659	Penicillin, 10 µg)
Escherichia coli	ATCC 11775	Tetracycline, 30 µg)
Aspergillus niger	ATCC 16404	
Streptococcus mutans	ATCC 25175	Penicillin, 10 µg)
Enterococcus faecales	ATCC 19433	Penicillin, 10 µg)
Methicillin-resistant *S. aureus* (MRSA)	ATCC 43300	Oxycillin, 1 µg)
Mycobacterium phlei	WARDS 85W1691	Ciprofloxacin, 5 µg)

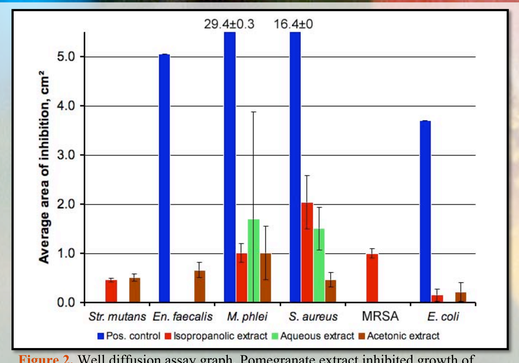

Figure 2. Well diffusion assay graph. Pomegranate extract inhibited growth of gram-negative and gram-positive bacteria, but not fungi. Isopropanolic extracts showed the greatest range of inhibition against various species. Error bars indicate variability within 5%. Neg controls (water, acetone, ispropanol solvents) did not inhibit the bacteria. Average of three trials. Error bars = 1 S.D.

Results

- *P. granatum* extracts showed the greatest inhibition against *Staphylococcus aureus.*
- Isopropanolic and acetonic extracts displayed inhibitory effects against the largest variety of screened organisms (**Figure 2**).
- The aqueous extract of *P. granatum* showed its widest range of antimicrobial efficacy after heating to 56°C with an average inhibition zone of 1.57 cm^2. The isopropanolic extract showed its widest range of antimicrobial efficacy after heating to 25°C with an average inhibition zone of 2.37 cm^2 (**Figure 3**).
- Disk diffusion assays were performed using disks cut from the alcoholic chromatogram. Disks at R_f values 0.01 and 0.07 inhibited *S. aureus* growth (**Figure 4**).
- The minimal inhibitory and bactericidal concentration against *S. aureus* was 125 mg/mL.
- Isopropanolic extracts inhibited antibiotic-resistant *Streptococcus mutans*; acetonic extracts inhibited MRSA (**Figure 5**).

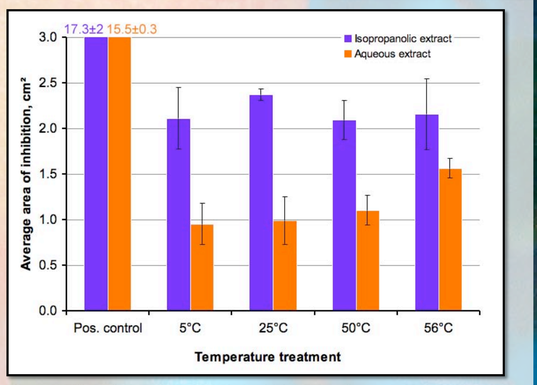

Figure 3. Effect of temperature on antimicrobial activity of extracts. Negative controls (water and isopropanol solvents) did not inhibit the bacteria. Average of three trials. Error bars = 1 S. D.

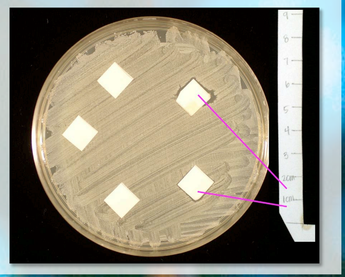

Figure 4. Isolation of the active compound. Compounds in isopropanolic extract were separated using paper chromatography. Disks 1 and 2 inhibited *S. aureus* growth.

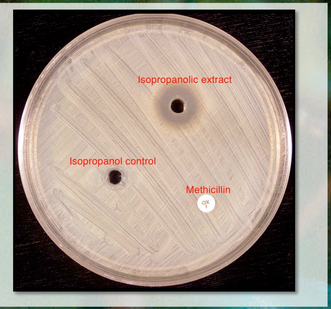

Figure 5. Well diffusion assay of pomegranate extract against methicillin-resistant *S. aureus*.

Discussion & Conclusion

- *P. granatum* has biologically active compounds that may be useful to treat gram-positive bacteria, *S. aureus*.
- MRSA is highly resistant to the antibiotics commonly used to treat staphylococcal infections. *P. granatum* may provide an alternative treatment for antibiotic-resistant *S. aureus.*
- *P. granatum*'s inhibitory properties may be useful to preventing food intoxication due to growth of *S. aureus* in food.

Literature Cited

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