Pome “Grenade” - Antimicrobial potency of pomegranate peel extract against *Streptococcus mutans*

Shashidara Raju, Sudheendra Udyavara Sridhara, Aparna H. Gopalkrishna, Vanishri C. Haragannavar, Archana Vaikom Krishnan

Department of Oral Pathology and Microbiology, Coorg Institute of Dental Sciences, Virajpet, Karnataka, India

**Abstract**

**Background:** Plant products have emerged in the forefront of management of several infectious diseases. Use of such therapeutically effective natural products generally entails lesser side effects and drug resistance. *Streptococcus mutans* is implicated in the common oral diseases including dental caries.

**Aim:** The present study is an attempt to investigate the anti-streptococcal activity of pomegranate peel extract.

**Materials and Methods:** Hydro-methanolic extract of fresh ground paste of fruit peels were prepared using a soxhlet apparatus and employed in an antimicrobial assay using a well diffusion and micro-broth dilution technique.

**Results:** All concentrations of the extract used show promising results with both antimicrobial assays used. The extract was effective in inhibiting the growth of *S. mutans* at concentrations as low as 1:8 diluted from the stock obtained after rotary evaporation.

**Conclusion:** The results implicate that exploring the application of *Punica granatum* extracts in oral health care could prove worthwhile. Further studies supported with in vivo evidence could implicate the extract to be an effective tool in oral health management such as mouthwashes, root canal irrigants, etc.

**Keywords**
Pomegranate, *Punica granatum* peel, *Streptococcus mutans*

**Introduction**

Antimicrobial drug resistance in human bacterial pathogens is a worldwide issue and as a consequence effective treatment and control of such organisms remains an important challenge. Bacterial resistance has appeared for almost every class of antibiotic and over the last decade research into antimicrobial properties of medicinal plants has become popular.\(^1\)

Pomegranate fruits are widely consumed both fresh and as commercial preparations such as jams, juices, and wines. They are one among the oldest known edible fruits that have been associated with fertility in the Bible and the Quran. Native to Persia, it gradually spread to being cultivated in Asia, North Africa and Mediterranean Europe including Turkey.\(^2\) The medicinal use of this plant dates back to 1500 BC where its use in parasitic infections by Egyptians has been elucidated in Ebers’ papyrus - one of the oldest medical writings. It was considered by them as a symbol of prosperity and ambition and used in sarcophagi decorations. The fruit has traditionally been used in curing acidosis, dysentery, microbial infections, diarrhea, helminthiasis, hemorrhage, and respiratory illness. It has also been used as an effective antiviral against herpes virus and influenza virus.\(^3\)

The peel of the fruit which accounts for about 50% of its total weight is not consumed and in industry accounts to a lot of wastage. The pomegranate peel has been extensively used in folklore medicine. Over the past years, researchers have shown that bio-active components of the peel possess anti-inflammatory, antioxidant, anti-atherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities.\(^4,5\) These functional components could be isolated and used in food enrichment and in the pharmaceutical industry to develop newer antimicrobials.\(^3\)

In the present study, we have used pomegranate peels and its hydro-methanolic extract in evaluating its antimicrobial property against the most common oral pathogen implicated in dental caries and one which is a key organism of the oral biofilm - *Streptococcus mutans*.\(^\)
Materials and Methods

Preparation of the extract
The extract was prepared from commercially available pomegranate purchased at a local market in Virajpet, Coorg district of Karnataka. The fruit peels were separated from the seeds and arils, roughly grated and ground in a mixer to yield a yellow rough paste. The paste was subjected to extraction with 30% methanol in distilled water with a soxhlet apparatus. Following this, the dilute extract was concentrated using a magnetic stirrer to yield a thick brown paste that was used in the antimicrobial assay.

Microbial strains and culture methods used
*S. mutans* strains ATCC 25175 (test organism) (HiMedia, Mumbai) were used in the study. The strains were subcultured and maintained on Mitis Salivarius agar (MSA) supplemented with 1% potassium tellurite. Prior to testing 24 h cultures were grown in Muller-Hinton broth (MHB) at 37°C ± 2°C under aerobic conditions and adjusted on a McFarland scale of 0.5 in the same broth prior to testing of the extract on them.

Assessing antimicrobial activity
Preliminary testing with the dilute extract before concentrating was carried out against *S. mutans* by the well diffusion technique on MSA plates. Following concentration further susceptibility testing was carried out using the micro-broth dilution technique and validated by well diffusion assay.

For the well diffusion assay, MSA plates were prepared and swabbed with 0.5 McFarland adjusted inoculum, following which wells were cut into them using a 5 mm borer. The concentrated extract was serially diluted using dimethyl sulfoxide as a solvent and 50 µl of each concentration of the extract was then dispensed into individual wells. Following this, the plates were incubated for 24 h at 37°C ± 2°C under aerobic conditions.

For the micro-broth dilution assay, 50 µl of each dilution was dispensed in 96 well micro-titer plates along with 50 µl of the McFarland adjusted strains of *S. mutans* in MHB as triplicates. Controls of just plain broth were also added as triplicates to be used in calculating the final inhibition values that would be obtained as a level of absorbance read at 630 nm. Amoxicillin at a concentration of 0.25 µg/ml was taken as positive control. The plates were incubated overnight for 24 h at 37°C ± 2°C. Following this, the supernatant of the wells were dispensed into a new plate before obtaining the optical density readings with a microplate reader (LabLife ER 2007), as the inherent consistency and color of the extract would hinder with readings.

Results
The preliminary testing by well diffusion against *S. mutans* showed promising results of a 2.3 cm zone of inhibition as compared to no inhibition seen on a control plate with 30% methanol [Figure 1], assuring that the inhibition was probably due to the pomegranate peel constituents and was under no influence of the solvent. Further well diffusion results are tabulated in Table 1.

The absorbance values obtained through micro-broth dilution technique showed a steady decrease in turbidity with increasing concentration of the extract corresponding to the decrease in bacterial load in the dispensed test solutions, which is represented in the form of a line graph in Figure 2.

Discussion
The antimicrobial potency of the parts of the pomegranate plant has been investigated on through many studies. The unused

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Zone of inhibition (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>2.3</td>
</tr>
<tr>
<td>1:2</td>
<td>2.8</td>
</tr>
<tr>
<td>1:3</td>
<td>2.6</td>
</tr>
<tr>
<td>1:4</td>
<td>2.6</td>
</tr>
<tr>
<td>1:5</td>
<td>2.6</td>
</tr>
<tr>
<td>1:6</td>
<td>2.7</td>
</tr>
<tr>
<td>1:7</td>
<td>2.6</td>
</tr>
<tr>
<td>1:8</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*S. mutans: Streptococcus mutans*

Figure 1: (a) Control plate with 30% methanol showing no inhibition with the solvent used, (b) test plate with dilute pomegranate extract showing a zone of inhibition - 2.3 cm

Figure 2: A steady decrease in bacterial load toward higher concentrations
Table 2: Phytochemical constituents of P. granatum

<table>
<thead>
<tr>
<th>Part</th>
<th>Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juice</td>
<td>Anthocyanins, glucose, ascorbic acid, ellagic acid, gallic acid, caffeic acid, catechin, EGCG, quercetin, rutin, numerous minerals, particularly iron; amino acids</td>
</tr>
<tr>
<td>Seed oil</td>
<td>95% punicic acid; other constituents, including ellagic acid; other fatty acids; steroids</td>
</tr>
<tr>
<td>Pericarp (peel)</td>
<td>Phenolic punicalagins; gallic acid and other fatty acids; catechin, EGCG; quercetin, rutin and other flavanols; flavones, flavonones; anthocyanidins</td>
</tr>
<tr>
<td>Leaves</td>
<td>Tannis (punicalin and punicafolin); and flavones glycosides, including luteolin and apigenin</td>
</tr>
<tr>
<td>Flower</td>
<td>Gallic acid, ursolic acid; triterpenoids, including malininc and asatic acid; other unidentified constituents</td>
</tr>
<tr>
<td>Roots and barks</td>
<td>Ellagittans, including punicalin and punicafolin; numerous piperidine alkaloids</td>
</tr>
</tbody>
</table>

P. granatum: Punica granatum, EGCG: Epigallocatechin gallate

peel of the plant has shown to contain numerous phenolic components, gallic acids, tannins, etc., [Table 2] [1,3,6] and this makes it the ideal plant component to harvest in assessing antimicrobial activity against oral bacteria, regarding which a few studies have been conducted. [7] In our study, at all concentrations used pomegranate peel extract was effective in inhibiting the growth of S. mutans. The same effects have been elucidated in other studies also where the peel extracts proved effective against various pathogenic organisms.[1,8,9] Other studies have also supported the use of methanolic extracts as used in our study showing the superiority of methanolic extracts of the seeds over the aqueous ones.[5]

Traditionally, the well diffusion method has been employed to evaluate antimicrobial activity. The micro-broth dilution technique as used in the present study is a viable alternative, especially when extracts are extremely viscous in nature. This technique also allows for maximum contact of extract directly with the organisms giving us real time results. The results of micro-broth dilution may be validated using a subsequent spread plate validation of each test well to check for microbial growth or parallel well diffusion assay. The dose-dependent bactericidal effect of the extract was elucidated by the micro-broth technique in this study [Figure 2].

With the proven in vitro effects of pomegranate peel extract on pathogenic bacteria, especially those of the oral cavity, the next step would be the incorporation of its active components into oral hygiene products. Few researches have shown the in vivo efficacy of the pomegranate plant extracts.[10]

The potential drawbacks of using the pomegranate peel components in oral formulations would be xerostomia due to the astringent nature of the fruit and bitter taste. Using suitable vehicles and including the purified active components of the peel could eliminate these drawbacks warranting its use effectively.

Conclusion

The results obtained in our study show the potency of pomegranate peel components as antimicrobial against S. mutans. With the peel practically having no known use, these results provide an exciting prospect of its use in the pharmacological industry. The effects of peel extracts against S. mutans and dental plaque imply that active components of the peel could form constituents of mouthwashes, root canal irrigants, toothpastes, etc. Using these peels in effect could both reduce tonnes of unused plant material and maximize use of the fruit as a whole.

References
