Anti-microbial activity of *Punica granatum* on streptococcus in dental caries patients and healthy individuals: 
A comparative study

Vidushi Lalwani, Anila Koneru, M. Vanishree, M. Vardendra, Santosh Hunasgi, R. Surekha

Department of Oral Pathology, Navodaya Dental College, Raichur, Karnataka, India

**Abstract**

**Background:** Researchers today are driven toward the medicinal and therapeutic value of the natural products like *Aloe vera*, basil, turmeric, green tea, pomegranate (*Punica granatum*) to prevent dental caries. The aim of the present study was to determine the antimicrobial activity of pomegranate aril extract on streptococci in dental caries patients and healthy individuals.

**Materials and Methods:** The study group included 40 subjects; age comprised of 18-26 years 20 dental caries patients were taken as an experimental group (after observing the decayed missing filled teeth index) and 20 healthy individuals without caries as a control group. Two samples were collected from each subject. The collected sample was streaked on *Mitis Salivarius Agar*, which was incubated at 37°C for 24 h. The colony forming units (CFU) were counted using manual colony counter, and the data were analyzed using paired *t*-test and unpaired *t*-test.

**Results:** The results obtained after pomegranate swish in the experimental group showed a percentage reduction of 52.2% (*P* < 0.0001) and 33.8% (*P* < 0.0001) in control group for the CFU. The comparison of after sample between the experimental group and the control group was highly significant.

**Conclusion:** The result obtained in the study clearly demonstrates that the pomegranate aril extract has an antimicrobial effect against *Streptococcus*, thus acting as an anti-cariogenic agent.

**Keywords**

Dental caries, *mitis salivaris agar*, oral rinse, pomegranate aril extract, *Streptococcus*

**Introduction**

Dental plaque is a biofilm which consists of bacteria growing on the surface of the tooth intertwined in a polysaccharide matrix. The most common plaque-related infectious dental diseases are dental caries, gingivitis, and periodontitis.

DC is the most prevalent disease of mankind amongst all age group. In India, nearly 60-70% of the population are affected by dental caries. The streptococci species especially *Streptococcus mutans*, the leading microorganism in the etiology of dental caries has been appraised widely via many cross-sectional studies.

Since DC is an irreversible disease which can only be treated by means of restoration that is expensive and laborious, thus prevention at the initial step is more essential. One of the most satisfactory possibilities for the control of DC involves the topical applications of fluoride with chemotherapeutic agents and reduces microorganisms from teeth. The widespread use of commercially available antimicrobials has led to the advent of multi drug resistant pathogens which ultimately lead a threat to global public health.

The use of plants for treating diseases is as old as the human civilization. There are many plants which have been in use as traditional medicine, so they are called as medicinal plants. The drill of plants for curing diseases was inevitable as is already proven by seeing the problems associated with synthetic antibiotics. *Punica granatum* L. (pomegranate), an ancient, magical, and distinctive fruit, is the predominant member of punicaceae family. According to ayurveda, it is considered “A pharmacy unto itself.” Various components of the plant such as the roots, bark leaves, flower, and extracts of the fruit including the seed oil, pericarp, and fruit juice have been used. According to Lansky, it is an extraordinary fruit with ample medicinal power contained within its peel, seeds, and juice.
Pomegranate juice contains polyphenols, tannins ellagic acid, and anthocyanin which are powerful antioxidants.\(^1\) Thus, the red arils (anthocyanin) having antioxidant property has a higher bioactivity compared to its purified polyphenols.\(^2\) As a result, pomegranate juice has become popular worldwide. Pomegranate preparations are used as topical applications particularly for controlling oral inflammation, as well as bacterial and fungal counts in periodontal disease and candida-associated denture stomatitis.\(^3\)

Epidemiological studies have suggested pomegranate (having antibacterial properties) which are true antibiotics as they are available for no cost, have no side effects and the most important benefit is that multi drug resistant pathogens will be easily killed by these new and natural antimicrobials because they will take at least a few decades to get mutated and resistant to them.

In the literature, very few studies have been carried out using pomegranate arils extract in dental caries and hence the aim of our study was to determine the antimicrobial activity of pomegranate aril extract on streptococci in dental caries patients and healthy individuals.

**Materials and Methods**

The present study is a clinical test conducted to find the effect of pomegranate aril extract on dental plaque microorganism (streptococci). Before the study was conducted, ethical clearance was obtained from Navodaya Dental College and Hospital. All the subjects were educated about the nature of the study.

The study group consists of 40 subjects in total, 20 dental caries patients (experimental group) and 20 healthy individuals without caries (control group).

**Inclusion criteria**

- The DC was considered after decayed missing filled teeth index was noted based on WHO diagnostic criteria, >5 score was considered for the study.

**Exclusion criteria**

- Medically compromised individual
- Individuals taking antibiotics in last 3 months period
- Individuals undergoing orthodontic treatment.
- Preparation of pomegranate aril extract: Pomegranate fruits were obtained from the arcade of Raichur, Karnataka, India. After washing, the peel/rind was removed, and the pulp of the fruit was separated from the seed by hand crushing method and filtered.
- Method of plaque collection: Two dental plaque samples were collected by swabbing all surfaces of the teeth from each subject using sterile cotton tipped swab. The first sample was taken after a saline rinse, and the second sample was taken 1 min after subjecting them to pure pomegranate aril extract which was swished for 2 min and spit. Each swab was placed in a 5 mL sterile container containing 2 mL normal saline (NS) and stored at 4°C until plated. All samples were plated within 12 h after collection. All swabs in NS were vortexed (2000 rpm) for 5 min to dislodge bacteria, and 0.004 mL was streaked on Mitis Salivarius Agar plate (Hi media), incubated at 37°C for 24 h.\(^{10,11}\) Bluish pinpoint 0.5 mm Colony forming units (CFU) were counted using manual colony counter\(^12\) (Figures 1 and 2). Gram staining and catalase test was conducted to confirm the colonies.

**Statistical analysis**

Data were expressed in mean and standard deviation. Comparison between before and after rinse for each group.
Activity of *Punica granatum* in dental caries patients

Lalwani, et al.

96


was done using paired *t*-test. Percentage of reduction was calculated for each group. Comparison between control and experimental groups was done by unpaired *t*-test. A *P* < 0.05 was considered as significant, and <0.01 was considered as highly significant. Data were analyzed by software Minitab v. 14.0. (Minitab® 14.0 Statistical Software, Minitab Inc. State College, United States of America).

**Results**

The mean numbers of (1 CFU = 10⁴ colonies/mL) of dental plaque microorganisms streptococci before and after rinsing pomegranate aril extract in the experimental group was 13.11 ± 5.81 and 6.27 ± 4 and in the control group it was 16.85 ± 8.05 and 11.15 ± 7.37, respectively. The percentage reduction in the number of colony forming unit was 52.2% (*P* < 0.0001) in experimental group and 33.8% (*P* < 0.0001) in control group. Both were highly significant [Table 1, Graphs 1 and 2].

The mean difference of CFU of dental plaque microorganisms streptococci in the experimental group and control group before rinsing was 3.74 and after rinsing pomegranate aril extract was 4.87, respectively [Tables 2 and 3].

**Discussion**

*P. granatum* most commonly known as pomegranate is a shrub native from Asia. It is an ancient fruit which has not changed much throughout the history of mankind. According to the Greek mythology, it was known as “fruit of the dead,” the Babylonians address it as “agent of resurrection” and the Chinese call it as a “soul concentrate” as it is homologous to human blood capable of conferring on a person longevity or immortality.[13]

The antimicrobial effects of pomegranate were previously studied. It is reported that the bark, leaves, flowers, and fruits of pomegranate are widely used as phytotherapeutic agent.[15] Pomegranate is a potent anti-oxidant[16] with anticarcinogenic and anti-inflammatory properties [Figure 3]. The biochemical constituent that makes it therapeutically beneficial are ellagic acid, ellagatannins, (including punicalin and punicalagin) punica acid, flavonoids, anthocyanins, estrogenic flavonols, flavonols and flavones.[9] There are many reports

<table>
<thead>
<tr>
<th>Group</th>
<th>Before (Mean±SD)</th>
<th>After (Mean±SD)</th>
<th>Mean difference</th>
<th>Percentage of reduction</th>
<th>95% CI</th>
<th><em>t</em> value</th>
<th><em>P</em> value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>16.85±8.05</td>
<td>11.15±7.37</td>
<td>5.71</td>
<td>33.8</td>
<td>3.47-7.94</td>
<td>5.34</td>
<td>&lt;0.0001</td>
<td>HS*</td>
</tr>
<tr>
<td>Experimental group</td>
<td>13.11±5.81</td>
<td>6.27±4</td>
<td>6.84</td>
<td>52.2</td>
<td>4.64-9.05</td>
<td>6.49</td>
<td>&lt;0.0001</td>
<td>HS*</td>
</tr>
</tbody>
</table>

**Table 1:** Efficacy of pomegranate extracts for with and without dental caries

**Table 2:** Comparison of control and experimental groups before rinse

<table>
<thead>
<tr>
<th>rinse ×10⁴ CFU/ML (Mean±SD)</th>
<th>Control group</th>
<th>Experimental group</th>
<th>Mean difference</th>
<th>95% CI</th>
<th><em>t</em> value</th>
<th><em>P</em> value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.85±8.05</td>
<td>13.11±5.81</td>
<td>3.74</td>
<td>0.75-8.23</td>
<td>1.68</td>
<td>0.1</td>
<td>NS**</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Comparison of control and experimental groups after rinse

<table>
<thead>
<tr>
<th>rinse ×10⁴ CFU/ML (Mean±SD)</th>
<th>Control group</th>
<th>Experimental group</th>
<th>Mean difference</th>
<th>95% CI</th>
<th><em>t</em> value</th>
<th><em>P</em> value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.15±7.37</td>
<td>6.27±4</td>
<td>4.87</td>
<td>1.07-8.67</td>
<td>2.59</td>
<td>&lt;0.01</td>
<td>Significant</td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard deviation, CI: Confidence interval, *NS*: Not significant, CFU: Colony forming units.

Graph 1: Comparison of before rinse and after rinse in experimental group and control group

Graph 2: The percentage of reduction in experimental group and control group
on antimicrobial activity of pomegranate showing that it is inhibitory effect on microbes.

The results obtained in the present study showed pomegranate aril extract have significantly higher inhibitory effect against Streptococcus in both dental caries patients and healthy individuals. The percentage of reduction was higher in experimental (52.2%) than control (33.8%). Comparison of after rinse values between experimental and control showed a statistically significant. Prashanth et al. also confirmed that methanolic extracts of pomegranate rind to be active against all microorganisms tested in their study. An in vitro study showed that Streptococcus sanguis, a bacterial strain present in the supra gingival plaque was sensitive to different concentration of pomegranate extract, which demonstrated inhibitory action similar to that of chlorohexidine.[15] Subramaniam et al. conducted an in vitro study and stated that hydroalcoholic extract of pomegranate aril showed significantly higher inhibitory effect on S. mutans at all concentration. Kote et al. stated that pomegranate rinse is effective against dental plaque microorganisms; there was a significant reduction in S. mutans colonies (23%).

What makes P. granatum anti-microbial? There are various schools of taught regarding the mechanism of action in which pomegranate act for the anticariogenic property First, the ellagitannin, punicalagin causes disturbance of polyglycans synthesis, and thus acts on the adherence mechanism of these organisms to the dental surface. Second, the tannin crosses over the cell wall of the microorganisms and binds to its surface leading to the precipitation of proteins and suppress enzyme like glycosyl transferase, the key enzyme for breakdown of carbohydrates. Third, the phenolic compounds bind to the substrate such as minerals and carbohydrate making them unavailable for the microorganisms to survive resulting in cell wall disruption [Figure 4].

Conclusion

The result obtained in the study clearly demonstrates that pomegranate aril extract has an antimicrobial effect against Streptococcus, thus acting as an anti-cariogenic agent. The presence of phyto-compounds: Phenols and tannins make pomegranate an efficient phyto-therapeutic agent. The potential use of pomegranate (oral rinse and tooth paste) as a convenient alternative to antimicrobial products has to be investigated, and long-term clinical trials should be conducted to know the safer dose that can be taken by humans.

Acknowledgment

I express my sincere thanks to Dr. Mahalaxmi, Department of Microbiology Navodaya Medical College, Raichur, Karnataka, India.

References
