

Chewing Pomegranate Fruit as an Adjuvant to Fight Against Early Childhood Caries

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Abstract: ***Aim:** Study was aimed to assess the effect of whole pomegranate fruit on oral bacterial load in children less than 6 years of age. **Materials and Methods:** 30 children between 4 to 6 years age were given 3 table spoons of whole pomegranate after collecting their baseline saliva sample. Children were instructed to abstain from food & liquids for 60 minutes. Post interventional saliva sample were collected after 60 minutes. Data was analysed by t-test and paired t-test at 0.05 level of significance. **Results:** The mean CFU/ml in the post - operative sample was significantly lower than in the pre - operative sample ($p < 0.05$). **Conclusion:** The whole pomegranate fruit has significant inhibitory effects on oral bacterial load, and is a promising adjuvant in preventing ECC.*

Keywords: Early childhood caries, oral bacteria, pomegranate

1. Introduction

Early childhood caries (ECC) is a significant–global health concern, impacting nearly half of preschool children worldwide, with a pooled prevalence of approximately 46.9%.¹ Alarmingly, one in every two children in India is affected by ECC. Untreated ECC can lead to severe pain, making it difficult for children to eat and speak, which adversely impacts their nutritional intake and overall development. As pedodontics, it is essential to combat ECC through early diagnosis, effective preventive care strategies. This includes decrease in bacterial load, increasing resistance of the tooth and reduction of substrate.

Biofilm is composed of a complex accumulation of microorganisms entrapped in a hydrated polymer matrix.² Microbial adhesion to tooth surface is probably the most important parameter responsible for biofilm formation.³ In children plaque elimination may not occur efficiently with mechanical plaque removal measures alone. One of the most effective antibacterial agents for preventing plaque is mouthwash. However, it has some side effects and complications such as tooth staining, altering the sense of taste, oral burning sensation, and interference with the oral microflora.^{4,5}

Herbal extracts have long been used for the treatment of infectious diseases.^{6,7} Nonetheless, research is still ongoing on the antimicrobial effects of plant extracts.⁸ A large body of evidence exists regarding the optimal antimicrobial properties of pomegranate skin and juice.^{9, 10} Pomegranate fruit has antioxidant properties. It eliminates the free radicals and chelates the ferrous ions. It also has anti-inflammatory and antibacterial effects, which are attributed to the constituents of the pomegranate peel, seed, and juice. The whole fruit has superior effects compared with only one component of the plant.¹¹ It is rich in hydrolysable tannins, which are polyphenols with polyol as their central core such as punicalagin/ ponicalin, ellagic acid, cyanidin-3-glucoside, cyanidin 3, 5-diglucoside, delphinidin, cyanidin, and pelargonidin (condensed tannins) as well as organic acids such as citric acid and ascorbic acid. Henceforth our study is aimed to assess the effect of whole pomegranate fruit on oral cariogenic bacteria.¹²

2. Materials & Method

Inclusion criteria:

- Cooperative children aged between 4 to 6 years
- No history of recent antibiotic administration (previous week) or anti - microbial mouth rinse (previous 12 hours).

Exclusion criteria:

- Children with systemic disease
- Children with special health care needs
- Children using any other oral hygiene aid other than routine teeth brushing
- Children with history of recent antibiotic administration or antimicrobial mouth rinse, or topical fluoride treatment.

Case selection:

30 children with informed consent based on inclusion exclusion criteria with clinical finding of ECC aged 4 to 6 years who visited outpatient department of KVG dental college, Sullia were included in the study.

Methodology:

Pre - operative salivary samples were collected from each child. 5 ml of unstimulated saliva was collected in a sterile container. Pomegranate (*Punica granatum*) were collected from the local public market. The Fruit was cleaned, then separated from the mesocarp and gathered in a bowl. Child was given 3 tablespoons of fruit to chew on both right and left sides and swallow. Participants were instructed to abstain from food & liquids for 60 minutes. Post interventional saliva sample were collected after 60 minutes.

Bacterial culture:

Pour plate method were used with Nutrient agar to culture the microorganisms. 1 litre of distilled water used to dissolve 28 gram of nutrient agar powder. 121 °C autoclaved for 15 minutes. Once the nutrient agar has been autoclaved, allow it to cool but not solidify. The saliva sample were diluted at 10⁻¹ and this sample is mixed with the molten agar medium prior to pouring. After pouring in the petri plate. The mixed medium is allowed to solidify and incubated at 37 °C for 24 hours. After incubation the isolated bacterial colonies were picked from growth plates and quadrant streaking done by rotating the plates at 90 ° anticlockwise at four different areas

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of plate. This was done by dragging the culture across the agar with help of sterilised inoculating loops from previously streaked area to new one. Then plate were incubated at 37 °C for 24 hours. The total viable oral microbial load were

examined and counted using microprocessor colony counter [CFU/ml].

3. Results

Table 1: Pre operative and Post operative sample

| Descriptive Statistics | | | | | |
|----------------------------------|----|------------------------|------------------------|-------------------------|-----------------------|
| | N | Minimum | Maximum | Mean | Std. Deviation |
| Age | 30 | 4 | 6 | 5.2 | 0.76112 |
| Pre - operative sample 1 CFU/ml | 30 | 345.00*10 ² | 679.00*10 ² | 549.038*10 ² | 85.88*10 ² |
| Post - operative sample 2 CFU/ml | 30 | 35.00*10 ² | 289.00*10 ² | 155.33*10 ² | 60.66*10 ² |
| Valid N (listwise) | 30 | | | | |

Study involved 30 participants, the age ranged from a minimum of 4 years to a maximum of 6 years, with a mean age of 5.2 years and a standard deviation of 0.76 years. Regarding the pre - operative sample, the concentration of CFU/ml ranged from 34, 500 to 67, 900 CFU/ml. The average concentration was 54, 903 CFU/ml, with a standard deviation of 8, 588 CFU/ml. For the post - operative sample, the CFU/ml concentration varied from 3, 500 to 28, 900 CFU/ml. The mean concentration was 15, 533 CFU/ml, with a standard deviation of 6, 066 CFU/ml.

Table 2: Gender distribution

| Gender | | | | | |
|--------|--------|-----------|---------|---------------|--------------------|
| | | Frequency | Percent | Valid Percent | Cumulative Percent |
| Valid | Male | 15 | 50 | 50 | 50 |
| | Female | 15 | 50 | 50 | 100 |
| | Total | 30 | 100 | 100 | |

Out of a total of 30 participants, 15 were male and 15 were female, making up 50% of the sample. The valid percent for both genders was 50%.

Table 3: Paired t - test comparison between pre and post operative samples

| Paired Samples Statistics | | | | | |
|---------------------------|----------------------------------|------------------------|----|-----------------------|---------|
| | | Mean | N | Std. Deviation | P Value |
| Pair 1 | Pre - operative sample 1 CFU/ml | 549.03*10 ² | 30 | 85.88*10 ² | 0 |
| | Post - operative sample 2 CFU/ml | 155.33*10 ² | 30 | 60.66*10 ² | |

For the pre - operative sample, the mean concentration was 54, 903 CFU/ml with a standard deviation of 8, 588 CFU/ml, based on 30 observations. The post - operative sample had a mean concentration of 15, 533 CFU/ml with a standard deviation of 6, 066 CFU/ml, also based on 30 observations. The p - value for the comparison between these two samples is 0.000, indicating a statistically significant difference between the pre - operative and post - operative concentrations.

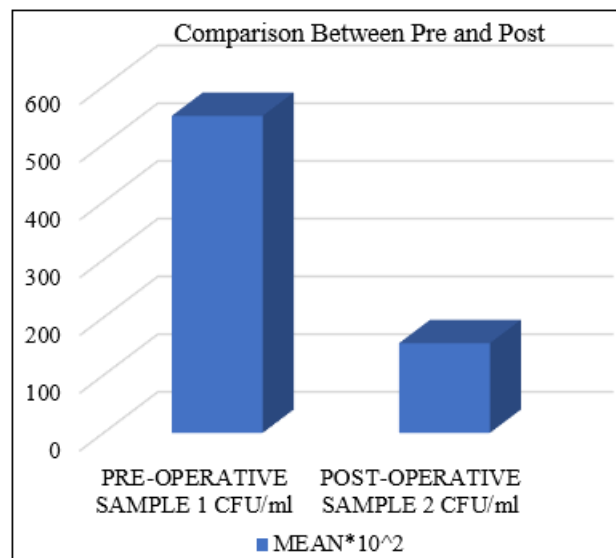


Figure 1

The mean CFU/ml in the pre - operative sample was significantly lower than in the post - operative sample (p< 0.05). This data implies that pomegranate consumption helps to reduce oral microbial load in young children.

4. Discussion

Pomegranate's active ingredients, such as polyphenolic flavonoids like ellagic acid and punicalagins, have been proven in a number of studies to potentially prevent gingivitis by a various mechanism, including the reduction of oxidative stress in oral cavity, direct anti - oxidant activity anti - inflammatory effects, antibacterial activity, and direct removal of plaque from the teeth.^{13 - 15}

Pomegranate suppresses the ability of these microorganisms to adhere to the surface of the tooth. Plaque formation can result from the combined action of four or more distinct microorganisms colonising the tooth surface. Remarkably, nature's own pomegranate fights the organisms' ability to adhere by interfering with the production of the very chemicals the bacteria use for adhesion.¹⁶

The total viable bacterial count was significantly lower after chewing pomegranate compared to pre op which is in accordance to an Ohio State study, those subjects who rinsed with pomegranate solution experienced a reduction in saliva total protein content which is normally higher among people with gingivitis and may correlate with plaque - forming bacterial content.¹⁷

This could be due to Pomegranate rinsing that lowered saliva activities of alpha - glucosidase, an enzyme that breaks down sucrose, while it increased the activities of ceruloplasmin, an antioxidant enzyme.¹⁸ Pomegranates may also have anticariogenic properties that could aid in prevention of dental caries.

Reddy *et al.*¹⁹ found that the antibacterial activity of the pomegranate fruit is due to its ellagic acid, gallagic acid, ponicalin, punicalagin, and tannin contents, which are effective against *Pseudomonas aeruginosa*, *Candida albicans*, methicillin-resistant *S. aureus*, *Aspergillus fumigatus*, and *Mycobacterium intracellulare*.²⁰

The pomegranate fruit has been used for centuries with no toxic effects.²¹ Pomegranate is a strong antioxidant with anticancer and anti-inflammatory properties. Several studies have confirmed its antimicrobial effects. Pomegranate has various antimicrobial ingredients and exerts antibacterial effects through several mechanisms. Punicalagin and ellagitannin impair the synthesis of polyglycans and prevent the adhesion of microorganisms to the tooth surface. Furthermore, tannins pass through the cell wall, adhere to the cell membrane, and cause protein deposition and suppression of enzymes such as glycosyltransferase. Moreover, phenolic compounds bind to the surface of substrates such as carbohydrates and minerals and make them unavailable to microorganisms, resulting in cell wall disintegration.²²

5. Conclusion

This study implies that pomegranate may be used as an adjunct to prevent ECC and maintain good oral hygiene by reducing the overall oral bacterial load.

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Picture 1: Bowl with 3 teaspoon of pomegranate aril



Picture 2: Salivary sample collection



Picture 3 – Child chewing pomegranate