

## Effect of Pomegranate Juice Extract on *Streptococcus mutans* - An In Vitro Study

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### ABSTRACT

#### BACKGROUND

Chlorhexidine the gold standard among anti-plaque agents, though one of the most potent antimicrobial agents, has several drawbacks bringing forth the need for studies focusing on development of new antibacterial agents that are equally or more effective with marginal consequences on the oral tissue. Pomegranate is known for its excellent antioxidant properties and antiplaque effect. Thus, this in-vitro study aims to estimate the influence of pomegranate juice extract mouth rinse (PJE) on *S. mutans* and compare the zone of inhibition of a pomegranate extract mouth rinse with chlorhexidine.

#### METHODS

Freshly prepared pomegranate juice was acquired using the sterilised grinder of the research laboratory. 400 mL of pomegranate juice was heated for 1 hr to get a heavy concentrate. Concentrations of 1200, 900, 600, 300, 150 and 75 mg / mL was tested. The bacterial strain expended in this study was *S. mutans* microbial type culture collection (MTCC) 890. Five samples of chlorhexidine was taken as control to compare the effect of pomegranate mouth rinse. Mann Whitney U test was employed to compare the antibacterial property between the two groups.

#### RESULTS

The zone of inhibition of *S. mutans* using PJE (300 and 600 mg / mL) was found to be statistically highly significant ( $P = 0.008$  and  $p = 0.007$ ) when compared to that of chlorhexidine.

#### CONCLUSIONS

PJE mouth rinse possesses remarkable antimicrobial activity against *S. mutans* present in the oral cavity as tested in vitro, and may be used as an adjunct to prevent dental caries and improve oral health.

#### KEY WORDS

Antibacterial, Mouth Rinse, pomegranate, PJE, *S. mutans*

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## BACKGROUND

Chlorhexidine the gold standard among anti-plaque agent, though one of the most potent antimicrobial agents have several drawbacks such as tooth discoloration, dysgeusia, and augmented calculus development.<sup>1</sup> Hence bringing forth the need for studies focusing on development of new antibacterial agents that are equally or more effective with marginal consequences on the oral tissue. *Streptococcus mutans* the major causative factor in dental caries, adheres to the tooth surfaces via water-insoluble glucan from sucrose and is responsible for biofilm formation.<sup>2,3</sup> Many studies have revealed that *S. mutans* represents about the 20 – 40 % of the cultivable flora in biofilms removed from carious lesion.<sup>4</sup> Even though the prevalence of dental caries has remarkably reduced in the past decade through the widespread preventive mechanisms such as use of fluoridated toothpastes and other fluoride containing agents, awareness regarding oral hygiene practices and sealants, it still remains one of the most common chronic diseases both in children and adults alike.<sup>5,6</sup> In this era of “turn to your herbal roots” both due to the beneficence of the herbal component and the lack of adverse effect, many herbal products are being studied and considered as antiplaque or antibacterial agents or as adjuvants. Among those herbal alternatives, pomegranate or *Punica granatum* is one of the most important mentions.

pomegranate is a common fruit widely used in traditional medicine worldwide. In various forms of traditional and oriental medicine, pomegranates are advocated as health supplements and as treatment modality for conditions like diarrhoea, dysentery and diabetes. It is most abundant in phytochemicals such as polyphenols such as the hydrolysable tannins called ellagitannins. Its red color is attributed to anthocyanins such as delphinidin, cyanidin and glucosides. It is used for treatment of various diseases due to its radical scavenging ability, ferrous ion chelating property and ferric ion reducing antioxidant power.<sup>7</sup> Numerous studies have been done on the antimicrobial properties of pomegranate peel and its curative effects.<sup>8-13</sup>

The antiplaque properties of pomegranate and its use in reducing the risk of gingival and periodontal conditions have been studied extensively. Various studies reports that the antioxidant property attacks the very source of tooth decay at a biochemical level.<sup>7,11,13,14,15,16-18</sup> Thus this study was conducted to estimate the influence of pomegranate extract mouth rinse on the *S. mutans* count and to compare the anti caries property of pomegranate extract with chlorhexidine mouth rinse.

## METHODS

An in-vitro study was conducted from 15/7/17 to 15 /10/17. Pomegranate juice extract was freshly prepared at the research laboratory using fresh pomegranate documented as *P. granatum* obtained from the local market. The whole fruit, including arils, seeds, peel, membrane and rind was cut into small pieces and ground to pulp using a sterilised grinder. It was then filtered initially using a muslin cloth followed by Whatman Grade 1 filter paper. 400 mL of the obtained pomegranate juice was then heated for one hour to get a heavy

concentrate. This was diluted to obtain the required concentrations of 1200, 900, 600, 300, 150, and 75 mg / mL.

A concentration of 75 mg / mL PJ mouth rinse was prepared by dissolving 4.5 g of PJE in 60 mL of distilled water. 150 mg / mL concentration of PJ was prepared by adding 9 g of PJE to 60 mL of distilled water. The 300 mg / mL concentration of PJ was prepared by dissolving 18 g of PJE in 60 mL of distilled water. 36 g of PJE was used to prepare the mouth rinse of 600 mg / mL concentration of PJ. A concentration of 900 mg / mL PJ was obtained by dissolving about 54 g of PJE in 60 mL of distilled water. 72 g of PJE was used to prepare the mouth rinse of 1200 mg / mL of PJ concentration.

## Microorganism and Media

The bacterial strain expended in this study was *S. mutans* microbial type culture collection 890. Mitis salivary agar was the culture media used.

## Methodology

The mitis salivary agar plates were spread with cultures of *S. mutans*. The five said concentrations of PJE were taken as test samples and 5 samples of 2 % chlorhexidine were taken as control to compare the effect of pomegranate mouth rinse. The plates were incubated at 37° C for 24 hours. The antimicrobial activity of PJE was assessed by measuring the zone of inhibition of *S. mutans* using agar well diffusion method.

## Statistical Analysis

The data collected was analysed using SPSS (Statistical Package for Social Sciences, Version 21). Descriptive statistics was calculated, including, mean standard deviation (SD) and median. Inferential statistics using Mann Whitney U test was employed to compare the mean zone of inhibition between the two independent groups. Level of significance was set at  $\leq 0.05$  and confidence interval was 95 %.

## RESULTS

An in vitro study was conducted to estimate the effect of pomegranate juice extract mouth rinse on *S. mutans* count and also to compare the antimicrobial effect of a pomegranate juice extract mouth rinse with chlorhexidine.

Mean zone of inhibition of *Streptococcus mutans* at different concentration of pomegranate juice extract (Table 1)

Table 1 shows that at 1200, 900, 150 and 75 mg / mL concentration of pomegranate juice extract, there were no zone of inhibition. Zone of inhibition were found in 600 and 300 mg / mL (1.94 cm and 1.42 cm) and standard deviation was found to be 0.19 and 0.13 respectively. Zone of inhibition of chlorhexidine mouth wash (control) was found to be 2.4 cm with a standard deviation of 0.070.

Comparison of mean zone of inhibition of pomegranate juice extract (300 and 600 mg / mL) with chlorhexidine in the inhibition of *Streptococcus mutans* (Table 2)

Table 2 shows that while comparing the PGE 600 mg / mL with chlorhexidine (control) the Z value was found to be -

2.652 and was found to be statistically highly significant ( $P = 0.008$ ). Comparing the PGE 300 mg / mL with chlorhexidine (control), the Z value was found to be - 2.677 and was found to be statistically highly significant ( $p = 0.007$ ).

Concentrations (mg / mL)	Mean Zone of Inhibition (cm)	Standard Deviation	Median
1200	0	0	0
900	0	0	0
600	1.94	0.19	2
300	1.42	0.13	1.5
150	0	0	0
75	0	0	0
CHX	2.4	0.07	2.4

**Table 1. Mean Zone of Inhibition of *Streptococcus mutans* at Different Concentrations of Pomegranate Juice Extract (PGE)**

Groups	Z	P Value
Chlorhexidine	- 2.652	0.008 *
Pomegranate juice extract 600 mg / mL	- 2.677	0.007*
Pomegranate juice extract 300 mg / mL		

**Table 2. Comparison of Pomegranate Juice Extract (300 and 600 mg / mL) with Chlorhexidine in the Inhibition of *Streptococcus mutans***

\*highly significant

## DISCUSSION

This study conducted to assess the antibacterial effect of PJE revealed zones of inhibition of *S. mutans* in two concentrations (600 and 300 mg / mL) however, the results were found to be statistically significant. The findings were in accordance with the results of the study conducted by Pereira<sup>19</sup> wherein inhibition was found at 150 mg / mL concentration and was not found to be statistically significant. Pereira concluded the study by saying that antibacterial activity of pomegranate can be attributed to polyphenolic flavonoids like punicalagins and ellagic acid present in them. Natural phenol such as ellagic acid is commonly found in numerous fruits and vegetables. However, punicalagins that are unique to the pomegranate are water-soluble, highly bioavailable, and supported by safety data. They possess a very high absorption rate of up to 95 % and have very powerful antioxidant properties of their own. They can further break down into smaller polyphenols known as urolithins that can also be absorbed and metabolised by the body. Another study reported by Umar et al<sup>13</sup> conducted on the effect of pomegranate mouth rinse on *Streptococcus mutans* count and salivary pH indicated a reduction in the number of *S. mutans* count 10 min and 60 min after oral rinse implying an inhibitory effect of PJE on bacterial growth thus concluding that pomegranate extract suppresses the ability of microorganisms to adhere to the surface of the tooth.<sup>13</sup> Pomegranate rinsing is reported to increase the activities of antioxidant enzyme ceruloplasmin in saliva while also lowering activities of alpha-glucosidase enzyme that breaks down sucrose.<sup>13</sup> This further contributes to the anti-cariogenic effects of pomegranate, which may be utilised to prevent dental caries in individuals. The other factors responsible for the inhibition of *S. mutans* according to literature can be attributed to punicalagins, tannin, ellagic acid and ceruloplasmin present in PJE. These hydrolysable tannins are antibacterial agents that can bind by crossing the microbial cell wall composed of several proteins and polysaccharides and precipitate proteins and suppress many enzymes such as glucosyl transferases. They also form complexes of high molecular weight with soluble proteins thus increasing

bacterial lysis and interfering with bacterial adherence.<sup>20</sup> The arils of the fruit contain lesser concentration of tannins compared to the outer carp. As we have included the whole fruit in our study, it will have its beneficence as well.

Vasconcelos et al and Lansky et al<sup>21,22</sup> conducted a study on minimum inhibitory concentration of adherence of *Punica granatum* extract against *S. mutans*. The result of the study indicated that *Punica granatum* extract was effective in inhibiting the adherence of the bacterial strains in the presence of sucrose. They concluded that when used regularly in combination with toothpaste, pomegranate containing mouthwash inhibits the activities of the microorganisms that cause plaque thereby may reducing dental plaque and tartar formation. Also, *Punica granatum* (pomegranate) juice extract had an inhibitory effect on the adherence of commonly found bacterium found in the oral cavity. Moreover, dental products containing natural substances have good market perspective and increased acceptance due to popular knowledge regarding lack of side effects as well as cultural or social values. Another factor increasing the acceptance is the awareness regarding the ill-effects associated with over prescription of antibiotics and the increasing resistance developed by micro-organisms against antimicrobials. Hence use of plants and plant derivatives which possess preventive and therapeutic effects to improve oral health can be utilised as alternative to conventional treatments and could be introduced in the dental market.<sup>23,24</sup>

The findings of the present study support the possibility that the *Punica granatum* (pomegranate) juice extract can be used in the control of bacteria responsible for oral infections such as caries. pomegranate containing mouthwash can also be used as an adjuvant with toothpaste to reduce the dental caries. Numerous studies have reported the effect pomegranate preparations to be effective in controlling inflammation, particularly oral inflammations as well as bacterial and fungal counts in periodontal disease and candida associated denture stomatitis. Hence, pomegranate is beneficial for oral health as well as overall health.<sup>25,26,21,27-30</sup>

## CONCLUSIONS

PJE mouth rinse possesses antimicrobial properties against *S. mutans* present in the oral cavity as tested in vitro, and may be used as an adjunct to prevent dental caries which might help to maintain good oral hygiene. Further investigations should be carried out to find compounds in pomegranate juice extract that help in the prevention of caries.

Data sharing statement provided by the authors is available with the full text of this article at jemds.com.

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