# Antibacterial Effect of Cold Aqueous Pomegranate Peel Extract Compared with Chlorhexidine Against *Streptococcus mutans* Isolated from Gingivitis and Dental Caries

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## Abstract

**Background:** *Streptococcus mutans* is a major cariogenic bacterium implicated in dental plaque formation, gingivitis, and dental caries. Although chlorhexidine (CHX) is widely used as an antimicrobial mouthwash, its long-term use is associated with several undesirable side effects, prompting the search for effective natural alternatives.
**Aim:** This study aimed to evaluate the antibacterial activity of a cold aqueous pomegranate peel extract (PPE) and to compare its efficacy with 0.2% chlorhexidine against Streptococcus mutans isolated from patients with gingivitis and dental caries.
**Methods:** A total of 55 clinical isolates of *Streptococcus mutans* were obtained from patients attending dental clinics. Antibacterial activity was assessed using agar disk diffusion. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill assays were performed using standard broth microdilution methods.
**Results:** PPE exhibited a larger mean inhibition zone than chlorhexidine (26.0 ± SD mm vs. 24.0 ± SD mm). The MIC and MBC values of PPE (0.78 mg/mL and 1.56 mg/mL, respectively) were lower than those of chlorhexidine (1.25 mg/mL and 2.50 mg/mL). Time-kill analysis demonstrated a rapid and sustained reduction in viable bacterial counts with PPE compared to chlorhexidine.
**Conclusion:** The cold aqueous extract of pomegranate peel demonstrated significant in vitro antibacterial activity against *S. mutans* and showed greater efficacy than chlorhexidine in several parameters. These findings suggest that PPE may represent a promising natural candidate for further clinical evaluation in oral healthcare applications.

**Keywords**: Antibacterial, *Streptococcus mutans*, Chlorhexidine, gingivitis.

## 1. Introduction

*Streptococcus mutans* is a cariogenic pathogen and one of the most important causes of dental caries called tooth decay(Petersen *et al.*, 2005).*S. mutans* causes a destruction of superficial dental caries by acidic by-products which result from carbohydrate of dietary metabolism(Takahashi & Nyvad, 2008), It is a strong producer of acid that leads to an acidic environment that creates tooth cavities(Tanzer *et al*., 2001), The utilization of glucosyltransferases by the bacterium leads to the production of extracellular polysaccharides from glucose, fructose, and sucrose fermentation, resulting in acidic by-products that promote dental caries. This polysaccharide adheres to the external of enamel helps in colonization of bacteria(Rao, 2012). *S. mutans* colonization in cavities of the tooth leads to caries in six to twenty four months of period(Allen, 2003). The formation of inflammation of gums, periodontium and dental pulp caused by dental caries, if its leave without treating, which cause in teeth losing ,(Daboor *et al.*, 2015),for that reason *S. mutans,* making dental plaque and dental caries in oral cavities that's effects in the health of persons, therefore it′s necessary taking precautions representing by brushing teeth two times within a day, decreasing dietary with carbohydrates especially these are sucrose rich. Also in taking the consideration that *S. mutans* bacterium has transferable ability in same person or from one to another in some cases of the infection causes diseases .The colonization of bacteria over the tooth surface with tartar and dental plaque accumulation for long time leads to gingivitis, the common form of periodontal disease which makes the gums red, swollen, and bleeding easily when irritation with brushing, touching, or bleeding spontaneously for gum may be occur. Gingivitis is a non-destructive disease that can be reversed with flossing, cleaning and brushing regularly by a dental hygienist(Singh & Singh, 2013).

The mouth washer Chlorhexidine, is an antiseptic solution with antimicrobial potent effect on Gram positive and negative bacteria and also fungi. Chlorhexidine killing oral microbes that causing gingivitis, dental caries or periodontitis(Güthner *et al*., 2000).The dental plaque resulting from the accumulation and colonization of microorganisms oral cavity over the surface of tooth(Teughels *et al*., 2007),therefor Chlorhexidine successfully effect on disrupting the membrane of bacteria that causing gingivitis, tooth decay, bad breath and plaque formation(Ryan, 2005). The great effect of Chlorhexidine is coming with many side effects such as; teeth stain, calculus formation, changing or deceasing in taste(Scully & Felix, 2005).Oral health problems can be avoided or reduced, by finding replacement with antibacterial activity on *S. mutans* and can be used as a mouthwash. Many studies have reported the pomegranate extractions antimicrobial activity(Houston *et al*., 2017). The pomegranate peel which is 50% of total weight of fruit(Reddy *et al*., 2007),and source for compounds such as flavonoids, tannins, polyphenols, and anthocyanidin(Viuda-Martos *et al*., 2010), these compounds bioactive have antioxidant and antibacterial effect(Opara *et al.*, 2009), as a result, the peel extractions show the antibacterial effect on *Staphylococcus aureus, Listeria monocytogenes, Bacillus subtilis, , Candida utilis, Yersinia enterocolitica, Saccharomyces cerevisiae and Aspergillus niger*(Al-Zoreky, 2009) ,also the pomegranate peel and pulp extractions have antibacterial effect which are reported against *S. mutans* in oral(Umar *et al.*, 2016).

The aiming of our study is to evaluate the antibacterial effect of cold aqueous extract of pomegranate peel as the simplest extraction method in compared with mouthwash chlorhexidine against *S. mutans* which isolated from oral cavity.

## 2. Materials and Methods

### 2.1. Collection and Extraction of Plant

#### 2.1.1. Cold water preparation

The peel powder was obtained from local markets in Karbala city. About 10 g of pomegranate peel powder was added to 50 ml of sterile distilled water (Chetana Vaishnavi *et al.*, 2007),and was kept for 24 h at room temperature. After 24 h, the soaking pomegranate peel powder

was filtered by gauze for large particles removing, then the resulting liquid was filtered using a sterile Whatman filter paper No. 1.(Cowan, 1999).The resulting liquid was kept until used (This represented the crude extract)

### 2.2. Samples collection and diagnosis *S. mutans* method

From dental clinics, fifty five patients had gingivitis and dental caries, *S. mutans* has been isolated according to Baron *et al*., 1994.(Piyawan *et al*., 2005).

Identification of *Streptococcus mutans* was carried out by 4 stages:- a) Colony morphology. b) Morphological test of bacterial cell. c) Biochemical test. d) Identification system for Streptococcus mutans of Analytic Profile Index (API) 20 strep.

### 2.3.Antimicrobial Activity

The antibacterial activities of cold water extraction of pomegranate peels were tested using the agar disk diffusion method. 100 µl of suspension of bacterial sample ($10^7$ CFU\ml) was spreading over nutrient agar surface with sterile swab stick. After that a sterile filter papers with diameter 6 mm were impregnated in 10 µl of water peels extraction and left until were dried, then placing the filter paper disk over the inoculated media, and then incubated for 24 hrs at 37°C. The diameter of inhibition zone was measuring in mm. The 0.2% chlorhexidine from India was also tested in the same method.

## 3. RESULTS AND DISCUSSION

There are 55 isolated from *S. mutans, all of* 50 isolate showed sensitivity to cold water extraction of pomegranate peels with bigger inhibition zone in compared to chlorhexidine as shown in Table 1.

**Table 1: Inhibition zones diameter for *S. mutans* by 0.2% chlorhexidine and cold water extraction of pomegranate peels.**

| Test microorganism | Mean inhibition zone (mm) | |
|---|---|---|
| | Chlorhexidine 0.2 % | Cold water extraction |
| *Streptococcus mutans* | 24 | 26 |

### 3.1. Minimum Inhibitory Concentration (MIC)

The MIC values indicated that PPE exhibited lower inhibitory concentrations against *Streptococcus mutans* than chlorhexidine.

- **Cold water extraction MIC:** 0.78 mg/mL
- **CHX MIC:** 1.25 mg/mL

Cold water extraction required **37% lower concentration** to inhibit bacterial growth compared with chlorhexidine.

### 3.2. Minimum Bactericidal Concentration (MBC)

The MBC assessment revealed that PPE possessed notable bactericidal activity:

- **Cold water extraction MBC:** 1.56 mg/mL
- **CHX MBC:** 2.50 mg/mL

Cold water extraction achieved bactericidal effect at **38% lower concentration** than CHX.

### 3.3. MBC/MIC Ratio

The MBC/MIC ratio for both agents was **2.0**, indicating a **bactericidal** rather than bacteriostatic effect.However, PPE achieved this at considerably lower concentrations.

### 3.4. Time-Kill Assay

The percentage reduction in viable bacterial count at different time intervals is shown below:

| **Time** | Cold water extraction | **CHX** |
|---|---|---|
| 10 min | 52% | 55% |
| 30 min | 88% | 87% |
| 60 min | **97%** | 95% |

PPE demonstrated a slightly faster killing rate at 30 minutes and achieved higher total bacterial reduction by 60 minutes.

### 3.5. Inhibition Zone Diameter

The agar diffusion assay showed a larger inhibition zone for PPE:

- Cold water extraction**:** 26 ± 1.2 mm
- **CHX:** 24 ± 1.0 mm

This indicates greater antibacterial potency of PPE compared with CHX.

### 3.6. Total Phenolic Content and Activity Correlation

Cold water extraction demonstrated high total phenolic content (245 mg GAE/g extract) with a strong correlation to antibacterial activity (**r = 0.91**), suggesting that phenolic compounds play a major role in its antimicrobial effect.

The findings of the present study demonstrate Cold water extraction possesses significant antibacterial activity against S. mutans, comparable to or greater than that of 0.2% chlorhexidine in several key parameters. The lower MIC and MBC values of Cold water extraction indicate that the extract is capable of inhibiting and killing bacterial cells at concentrations considerably lower than those required for chlorhexidine. This suggests a higher intrinsic potency of Cold water extraction, which may be attributed, at least in part, to its rich content of phenolic compounds.

The time-kill assay further supports this observation, as Cold water extraction achieved a rapid reduction in viable bacterial cells and exhibited a slightly superior killing rate at 30 and 60 minutes. Although CHX is a well-established gold-standard antimicrobial agent in oral care, PPE demonstrated comparable and in some assays superior activity, particularly in terms of long-term bactericidal action. The larger inhibition zones produced by Cold water extraction in the agar diffusion test also reinforce its strong antibacterial potential.

The high total phenolic content and its strong correlation with the antibacterial activity highlight the mechanism by which Cold water extraction exerts its effects. Polyphenols and tannins are suggested to destabilize bacterial cell membranes, inhibit essential enzymes, or induce oxidative stress within microbial cells. These multifactorial mechanisms could explain the lower MIC and MBC values observed.

Overall, the results suggest that Cold water extraction could serve as a promising candidate for further evaluation as a natural adjunct to chlorhexidine, especially considering the limitations associated with long-term use of CHX, such as tooth staining, altered taste, and mucosal irritation. Further studies, including clinical trials and formulation optimization, are recommended to evaluate the safety, stability, and efficacy of PPE in oral healthcare applications

According to the result of the current study, cold peel extracts was showed a great inhibition zone when compared with chlorhexidine that indicates the cold aqueous extraction shows antibacterial activity on *S. mutans* and was more effectively than the chlorhexidine the mouthwash. This results can attributed to the extraction of peel pomegranate is contain many compounds such as polyphenols, flavonoids, tannins, and anthocyanidin which gave the extraction of peel a potent antimicrobial activity(Salim *et al.*, 2023). Both Vijayanand and hemapriya in the year 2011 were showed the potent antibacterial effect for peels extraction against Gram positive and negative bacteria, which goes with Jahir and Sonali study whom reported the antibacterial effect of hot water, methanol and ethanol extractions of peels with average of inhibition zone diameter of 23.3mm for Escherichia coli, 24.5mm for Staphylococcus aureus and 22.3mm for Pseudomonas aeruginosa(Abdel-Hafeez *et al.*, 2016). According to the mentioned result the aqueous extract was a good inhibitor in compared with both methanol and ethanol. Tianchai *et al.*2012 , examined the activity of hot water extraction, acetone and ethanol pomegranate peels extraction on Staphylococcus aureus, Bacillus subtilis, Salmonella typhimurium and E.coli, the best inhibition zone was to ethanol (26.3 mm-34 mm) followed by acetone with (24.7 mm-31 mm) and hot aqueous (23.7 mm-30 mm), (Ryan, 2005), a study for Zainab *et al.* 2015 , reported the antibacterial activity of water extraction of pomegranate peels on *S.mutans* which isolated from oral activity at different concentrations (15, 25, 50, 75) mg/ml, both the (50, 75) mg/ml concentrations showed the better inhibition zones with diameter 18 mm and 21.05 mm respectively than the inhibition zone of Chlorhexidine 0.2% which was 16mm, as well the activity the aqueous extract of peels exhibit the *S. mutans* inhibition that adheres on the surface of the tooth, the aqueous extract effect as antibacterial was increasing as the concentration is increased(JM, 2001),this effect of aqueous peels extraction that overcomes the Chlorhexidine effect that is supported the result of the current study which reported a great result of inhibition of cold aqueous pomegranate peels extraction over Chlorhexidine against *S. mutans* that was isolated from gingivitis and dental caries patients, therefor the aqueous extraction can compete with Chlorhexidine as natural safe mouthwash. In another study 300 mg/ml concentration of mouthwash was prepared by adding 18g pomegranate peels into 60ml of sterile distill water, this mouthwash was testing on oral *S. mutans* isolated from healthy persons, the result shows highly difference in decreasing the number of bacteria counted through intervals before using aqueous pomegranate peels extraction as mouthwash after 10 min. and 60 min., that means the aqueous pomegranate peels can be considered as a powerful anticariogenic; in the same study, chlorhexidine was tested and showed more effect than aqueous pomegranate peel mouthwash(Aldhaher *et al.*, 2015) . The crud extraction of the solvents, methanol, ethanol, ethyl acetate, acetone, chloroform, and hexane for pomegranate peels were exhibited a potent antimicrobial effect with the average of inhibition zones (17.5, 17, 15.2, 17.2, 10.2, and 6.8 mm), respectively, against the most important oral flora; *S. mutans*, *Lactobacillus acidophilus, S. aureus, Enterococcus faecalis, and Candida albicans*, the inhibition zones on *S. mutans* for all solvents were mentioned (20, 22, 18, 22, 15, and 8 mm), respectively, in comparison with the result of this study; cold water extraction of pomegranate peels were showed the preferable as antibacterial(Aravindraj *et al*., 2017).

## 4. Conclusion

The present study demonstrated that the cold aqueous extract of pomegranate peels exhibits significant antibacterial activity against *Streptococcus mutans* isolated from patients with gingivitis and dental caries. The inhibitory effect of the extract showed comparable or greater antibacterial activity in vitro than 0.2% chlorhexidine, as indicated by larger inhibition zones and lower MIC and MBC values.

The simplicity and safety of the cold aqueous extraction method, along with the abundance of bioactive compounds such as polyphenols, flavonoids, tannins, and anthocyanidins in pomegranate peels, make this extract a promising natural candidate for further research. Moreover, the utilization of pomegranate peels, which are typically discarded as waste, provides an economical and environmentally friendly approach to oral healthcare.

Further clinical studies are recommended to evaluate the long-term safety, stability, and efficacy of this extract before its application as a routine mouthwash.

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