



www.bioinformation.net
Volume 22(8)



Research Article

Received August 1, 2026; Revised August 31, 2026; Accepted August 31, 2026, Published August 31, 2026

DOI: 10.6026/973206300225173

SJIF 2026 (Scientific Journal Impact Factor for 2026) = 8.478

2022 Impact Factor (2023 Clarivate Inc. release) is 1.9

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Citation: Rawat *et al.* Bioinformation 22(8): 5173-5178 (2026)

Efficacy of pomegranate extract enriched mouthwash versus chlorhexidine mouthwash for plaque and gingivitis control: Randomized clinical trial

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Abstract:

Chlorhexidine mouthwash has been associated with discoloration of teeth and altered taste. Hence, 20 participants were randomly divided into two groups: Group A pomegranate peel extract mouthwash; Group B 0.2% chlorhexidine mouthwash. The Loe-Silness Gingival Index and the Turesky-Gilmore Modified Quigley-Hein Plaque Index were assessed at baseline, day 14 and day 21. The group using pomegranate extract mouthwash showed a greater improvement in the gingivitis index on days 14 ($p = 0.009$) and 21 ($p < 0.05$). Pomegranate extract mouthwash was more effective in reducing inflammation of the gingival. It can be used as an adjunct therapy in the prevention of gingival diseases.

Keywords: Chlorhexidine (CHX), gingivitis, mouthwash, plaque, pomegranate, *P. granatum*

Background:

Gingivitis is a condition causing reversible inflammation of the gums primarily caused by plaque accumulation [1]. Although mechanical plaque removal methods are effective, some patients fail to adhere to them properly [2]. Therefore, chemical plaque control agents have gained significant importance in preventive dentistry. Chlorhexidine gluconate (CHX) is the gold standard in antimicrobial mouthwashes for decades, showing excellent efficacy against oral pathogens [3]. However, long-term use of chlorhexidine has been associated with several undesirable side effects, such as discoloration of teeth, altered taste, increased tartar buildup on gums and mucosal irritation [4]. These adverse effects have prompted researchers to explore other natural alternatives derived from medicinal plants. Pomegranate is rich in polyphenolic compounds, including punicalagin, ellagic acid, gallic acid and anthocyanins, which give it potent antioxidant, antimicrobial and anti-inflammatory properties [5, 6]. Previous phytochemical analyses have shown that pomegranate peel extract exhibits significant antibacterial activity against *S. mutans*, *P. gingivalis* and other oral tissue pathogens [7]. Several clinical studies have evaluated the effectiveness of pomegranate-based oral care products in treating gingivitis, with promising but varying results [8-10]. While some studies have shown similar or better anti-gingivitis effects than chlorhexidine [11], others have found CHX to be more effective in reducing plaque buildup [12]. Therefore, it is of interest to compare the effectiveness of a pomegranate peel extract mouthwash with a 0.2% chlorhexidine mouthwash in controlling plaque and reducing gingival inflammation.

Materials and Methods:**Study design:**

This randomized, double-blind, parallel-group clinical trial was conducted at the outpatient department of Public Health Dentistry, Teerthanker Mahaveer Dental College and Research Centre, Moradabad, Uttar Pradesh, India.

Ethics approval and consent to participate:

The study protocol was approved by the Institutional Ethics Committee (Ref. No. TMDCRC/IEC/SS/24-25/PHD) and registered with the clinical trials registry of India (CTRI/2025/09/095469) before participant enrollment. All procedures were in accordance with the ethical principles outlined in the Declaration of Helsinki (2013 version) [13]. Written informed consent was acquired from all participants after providing complete information about the study.

Sample size calculation:

The sample size was estimated using G*Power 3.1.9.7 software, based on a prior statistical power analysis. Assuming a large effect size (Cohen's coefficient $d = 0.85$), a two-tailed independent samples t-test, a significance level (α) of 0.05 and a required statistical power of 0.80, the calculated minimum sample size was 10 participants per group (total $n = 20$).

Participant selection:

Fifty outpatients underwent initial screening to determine their eligibility for the study.

Inclusion criteria:

Participants aged 18 to 40 years with mild to moderate gingivitis, having at least 20 natural teeth present, excluding the third molar, which were willing to comply with the study protocol and gave written informed consent.

Exclusion criteria:

Participants on antibiotics within past month; using antimicrobial mouthwash or participation in another oral hygiene study; intaking high dietary polyphenols; active orthodontic treatment; tobacco use in any form; presence of any systemic diseases; history of periodontal treatment in the past three months; pregnancy or breastfeeding; allergy to pomegranate or chlorhexidine; or suffering from advanced periodontal disease were excluded. Of the 50 screened

individuals, 22 did not meet the inclusion criteria and 8 declined to participate. The remaining 20 eligible participants were enrolled and randomly assigned to intervention groups (**Figure 1**).

Randomization and allocation concealment:

Participants were randomly assigned 1:1 to the pomegranate and chlorhexidine groups using a computer-generated random list (block size 4). This sequence was created by an independent researcher who was not involved in the enrollment process or evaluation of results. Allocation was kept confidential using sequentially numbered, sealed, opaque envelopes, which were opened only after participant eligibility was confirmed. Throughout the study, both participants and the examiner were blinded to the group distribution because the mouthwash bottles were labeled identically using an alphanumeric code.

- [1] Group A (n = 10, 7 males and 3 females): 0.05% *Punicagranatum* mouthwash
- [2] Group B (n = 10, 8 males and 2 females): 0.2% chlorhexidine mouthwash (REXIDINE)

Preparations of pomegranate peel extract mouthwash:

Fresh pomegranate fruits were bought from the local market. Peels were washed and then sun-dried for 48 hours followed by oven drying at 33°C for 5-6 days. Dried peels were ground into a fine powder and sieved (60-micrometer). To prepare the aqueous extract, 5 g of powder the peel was added to 250 ml of distilled water and heated in a water bath at 70°C for 60 min. The mixture was filtered using Whatman No. 1 filter paper and the filtrate was condensed using a rotary evaporator. The dried extract was dissolved in dimethyl sulfoxide to prepare a 50 mg/mL basic solution. This basic solution was diluted with distilled water (1:99 v/v) to obtain a final concentration of 0.05% pomegranate extract mouthwash, which was stored in amber-colored bottles at 4°C until use [14].

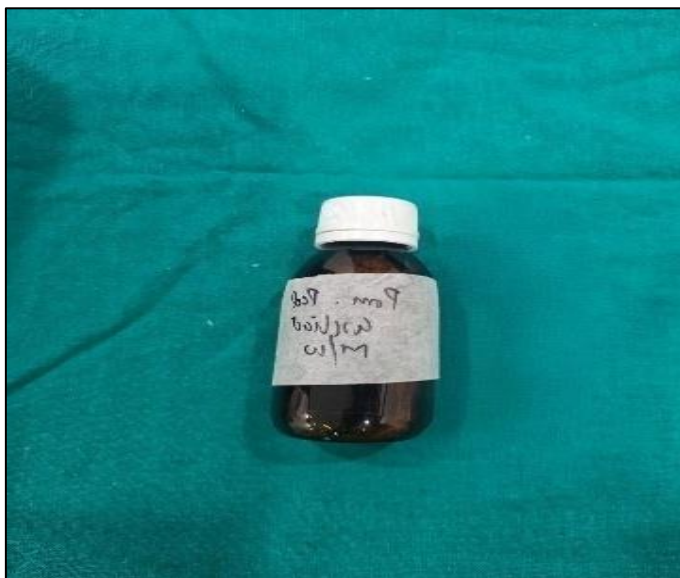


Figure 2: Pomegranate peel extracts mouthwash

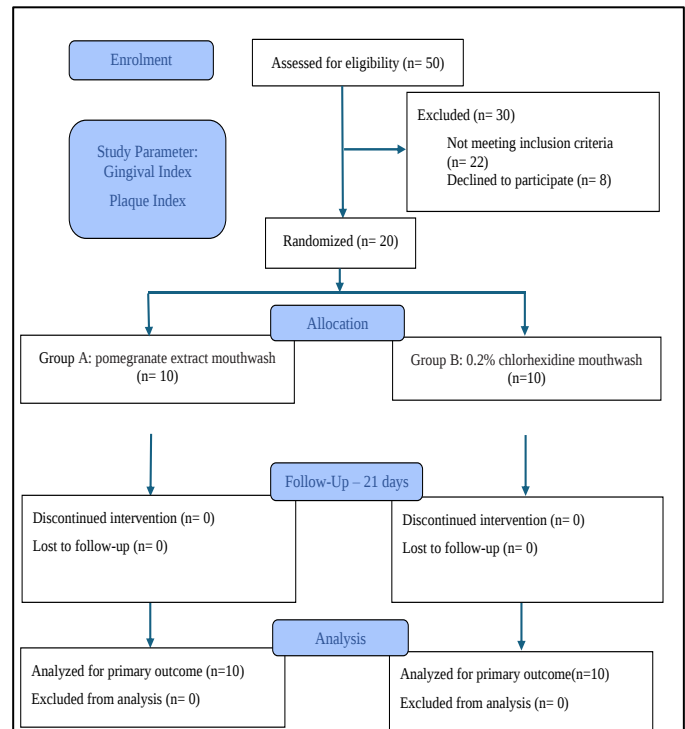


Figure 1: Consort flowchart

Intervention and compliance monitoring:

At the start of the study, all participants received oral hygiene instructions, including a practical demonstration of the modified Bass brushing technique. They were provided with identical soft-bristled toothbrushes and fluoride toothpaste to ensure consistency. Participants were instructed to brush their teeth twice a day and not use any other oral hygiene products. After the initial examination and brushing, participants received coded mouthwash bottles with instructions to use 10 ml of mouthwash for 30 seconds twice a day, 12 hours apart and to refrain from eating or drinking for 30 minutes after rinsing. To monitor adherence to these instructions, the amount of mouthwash remaining in returned bottles was measured during follow-up visits.

Clinical outcome measures:

All clinical assessments were performed by a single, calibrated examiner. Data was noted at the baseline followed by scaling at the same visit, day 14 and day 21.

The primary outcome measures were:

- [1] Gingival health assessed using Loe and Silness gingival index (GI) [15].
- [2] Plaque scores assessed using Turesky-Gilmore modification of Quigley Hein plaque index (PI) [16,17]

Statistical analysis:

Data were examined using IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, New York, USA). Descriptive statistics (means and standard deviations) were calculated for GI and PI

scores at each time point. The Shapiro-Wilk test confirmed normal data distribution ($p > 0.05$). Baseline demographic characteristics were compared using independent samples t-tests for continuous variables (age) and chi-squared tests for categorical variables (gender). Intergroup comparisons of GI and PI scores were performed using independent samples t-tests at each time point. Within-group changes across time points were examined using repeated-measures analysis of variance (ANOVA). Tukey's test for true significant difference was used for post hoc pairwise comparisons. Statistical significance was set at $p < 0.05$ for all analyses.

Results:

Twenty participants successfully completed the trial without any withdrawals or protocol violations. Group A consisted of 7 males and 3 females (**Table 1**) with an average age of 27.9 ± 4.7 years, while Group B consisted of 8 males and 2 females with an average age of 27.9 ± 3.1 years. Independent samples t-tests showed no significant difference in average age between the two groups ($t = 0.000$, $p = 1.000$) (**Table 2**), indicating successful randomization. Chi-square analysis also showed no significant difference in sex distribution between the two groups ($\chi^2 = 0.267$, $p = 0.606$). At the commencement of the study, the mean gingival index in group A was 1.06 ± 0.30 , while in group B it was 1.31 ± 0.22 . Although group B appeared to have slightly higher gingival inflammation at the beginning of the study, this difference approached but did not reach statistical significance ($t = 2.102$, $p = 0.051$). On day 14, the mean GI in Group A (0.57 ± 0.32) was notably lower than in Group B (1.04 ± 0.39), with a statistically significant difference ($t = 2.95$, $p = 0.009$). This trend intensified on day 21, with the mean gingivitis index reaching 0.36 ± 0.27 in group A, compared to 0.92 ± 0.37 in group B. The difference on day 21 was statistically significant ($t = 3.875$, $p = 0.001$), indicating a superior effect of pomegranate extract in reducing gingivitis during the 21-day intervention period (**Table 3**). The average score of baseline plaque index was 1.60 ± 0.85 in group A and 1.29 ± 0.52 in group B and there was no statistically significant difference between the two groups ($t = 0.973$, $p = 0.346$), confirming the similar level of initial plaque. On day 14, the mean PI for group A was 1.33 ± 0.78 , while group B had a lower mean PI of 0.78 ± 0.50 . Although CHX appeared more effective at reducing plaque, the difference approached but did not reach conventional statistical significance ($t = 1.873$, $p = 0.08$). On day 21, a significant difference was observed between the two groups, with the mean PI in group A being 1.18 ± 0.69 compared to 0.48 ± 0.34 in group B ($t = 2.871$, $p = 0.013$). These results indicate a superior effect of CHX compared to pomegranate extract in combating plaque by the end of the intervention period (**Table 4**). ANOVA for repeated measures revealed a statistically significant decrease in GI and PI scores in each group over the 21-day intervention period. In group A, the

mean gingival index gradually decreased from 1.06 ± 0.30 at baseline to 0.57 ± 0.32 on day 14 and then to 0.36 ± 0.27 on day 21 ($F = 83.148$, $p < 0.001$). Similarly, the mean plaque index decreased from 1.60 ± 0.85 at baseline to 1.33 ± 0.78 on day 14 and then to 1.18 ± 0.69 on day 21 ($F = 19.696$, $p < 0.001$). In group B, the mean GI decreased from 1.31 ± 0.22 at baseline to 1.04 ± 0.39 on day 14 and then to 0.92 ± 0.37 on day 21 ($F = 13.251$, $p < 0.001$). The mean PI showed an even greater decrease, from 1.29 ± 0.52 at baseline to 0.78 ± 0.50 on day 14 and then to 0.48 ± 0.34 on day 21 ($F = 38.865$, $p < 0.001$) (**Table 5**). Tukey's HSD test was used to determine two-way differences between time points within each group. In group A, a significant decrease in GI was observed from baseline to day 14 (mean difference = 0.490 , $p = 0.000$), from baseline to day 21 (mean difference = 0.700 , $p = 0.000$) and from day 14 to day 21 (mean difference = 0.210 , $p = 0.003$). Similarly, PI showed a significant decrease between all-time points ($p < 0.01$). In group B, the GI decreased significantly from baseline to day 14 (mean difference = 0.270 , $p = 0.022$), from baseline to day 21 (mean difference = 0.390 , $p = 0.002$) and from day 14 to day 21 (mean difference = 0.120 , $p = 0.001$). The PI also decreased significantly from baseline to day 14 (mean difference = 0.513 , $p = 0.001$), from baseline to day 21 (mean difference = 0.813 , $p = 0.000$) and from day 14 to day 21 (mean difference = 0.300 , $p = 0.001$) (**Table 6**). No side effects such as tooth discoloration, taste changes, mucous membrane irritation, or allergic reactions were reported in any group during the 21-day intervention period.

Table 1: Subject distribution according to gender in two groups

Group	Gender	Frequency	Percent
A	Male	7	70.0
	Female	3	30.0
B	Male	8	80.0
	Female	2	20.0

Table 2: Age wise comparison of subjects in two groups

Group	Mean	Std. Deviation	t value	p value
A	27.90	4.70	0.000	1.000
B	27.90	3.14		

Table 3: Inter group comparison of GI index values at different point of time

Time	Groups	Mean	Std. Deviation	t value	p value
Baseline	A	1.06	0.30	2.102	0.051
	B	1.31	0.22		
14 th day	A	0.57	0.32	2.95	.009*
	B	1.04	0.39		
21 st day	A	0.36	0.27	3.875	.001*
	B	0.92	0.37		

Table 4: Inter group comparison of PI index values at different points in time

Time	Groups	Mean	Std. Deviation	t value	p value
Baseline	A	1.60	0.85	0.973	0.346
	B	1.29	0.52		
14 th day	A	1.33	0.78	1.873	0.08
	B	0.78	0.50		
21 st day	A	1.18	0.69	2.871	.013*
	B	0.48	0.34		

Table 5: Intra group comparison of indices at different time points in two groups

Groups	Index	Time	Mean	Std. Deviation	F value	p value
A	GI	Baseline	1.06	0.30	83.148	<.001*
		14 th day	0.57	0.32		
		21 st day	0.36	0.27		
	PI	Baseline	1.60	0.85	19.696	<.001*

B	GI	14 th day	1.33	0.78	13.251	<.001*
		21 st day	1.18	0.69		
		Baseline	1.31	0.22		
		14 th day	1.04	0.39		

Table 6: Post-hoc comparison intra group

Groups	Indices	Time point		Mean Difference	Std. Error	p value	95% Confidence Interval for Difference	
							Lower Bound	Upper Bound
A	GI	Baseline	14th day	0.490*	0.053	0.000	0.371	0.609
			21st day	0.700*	0.061	0.000	0.561	0.839
		14th day	Baseline	-0.490*	0.053	0.000	-0.609	-0.371
			21st day	0.210*	0.053	0.003	0.091	0.329
		21st day	Baseline	-0.700*	0.061	0.000	-0.839	-0.561
			14th day	-0.210*	0.053	0.003	-0.329	-0.091
	PI	Baseline	14th day	0.270*	0.063	0.002	0.127	0.413
			21st day	0.420*	0.088	0.001	0.221	0.619
		14th day	Baseline	-0.270*	0.063	0.002	-0.413	-0.127
			21st day	0.150*	0.045	0.009	0.047	0.253
		21st day	Baseline	-0.420*	0.088	0.001	-0.619	-0.221
			14th day	-0.150*	0.045	0.009	-0.253	-0.047
B	GI	Baseline	14th day	0.270*	0.098	0.022	0.049	0.491
			21st day	0.390*	0.089	0.002	0.189	0.591
		14th day	Baseline	-0.270*	0.098	0.022	-0.491	-0.049
			21st day	0.120*	0.025	0.001	0.064	0.176
		21st day	Baseline	-0.390*	0.089	0.002	-0.591	-0.189
			14th day	-0.120*	0.025	0.001	-0.176	-0.064
	PI	Baseline	14th day	0.513*	0.099	0.001	0.290	0.736
			21st day	0.813*	0.111	0.000	0.562	1.064
		14th day	Baseline	-0.513*	0.099	0.001	-0.736	-0.290
			21st day	0.300*	0.063	0.001	0.157	0.443
		21st day	Baseline	-0.813*	0.111	0.000	-1.064	-0.562
			14th day	-0.300*	0.063	0.001	-0.443	-0.157

Discussion:

The study provides strong evidence that pomegranate peel mouthwash is highly effective in reducing gingival inflammation and plaque accumulation, although its effectiveness differs from that of chlorhexidine. Our results show that pomegranate extract is highly effective against gingival inflammation, but less effective against plaque than chlorhexidine over a 21-day intervention period. The higher anti-gingivitis effect noticed with pomegranate extract is consistent with previous studies. Sasi *et al.* [18] and Deepak and Samuel [19] exhibited similar or better anti-inflammatory effects than chlorhexidine. The enhanced anti-gingivitis property of pomegranate extract may be attributed to its rich composition of polyphenolic compounds, particularly punicalagin and ellagic acid, which exhibit potent anti-inflammatory properties [20]. The anti-inflammatory mechanism of pomegranate extract involves multiple pathways. Delphinidin inhibits inflammatory mediators, including NF-κB, COX-2 and interleukins [21]. Furthermore, the polyphenols in pomegranate exhibit antioxidant activity, scavenging reactive oxygen species that damage gum tissue during inflammation [22]. The astringent properties of the tannins may promote tissue repair and reduce bleeding from the gingiva by constricting blood vessels and precipitating mucosal surfaces [23]. In contrast, chlorhexidine exhibited superior plaque reduction by day 21, consistent with its well-known mechanism. The cationic chlorhexidine molecule binds to negatively charged cell walls of bacteria and oral mucosa, disrupting membranes and preventing bacterial adhesion to tooth surfaces [24]. Its long-lasting effect maintains antimicrobial effects up to 12 hours after rinsing [25]. Similarly, Kaur *et al.* reported superior plaque-fighting effects of

chlorhexidine compared to herbal alternatives, including mouthwashes containing catechins found in green tea [12]. Our results somewhat contradict other studies that have suggested similar anti-plaque effects of pomegranate. While Overholser *et al.* and Haffajee *et al.*, found no significant difference between herbal and chlorhexidine mouthwashes [26, 27], Menezes *et al.* found a superiority of pomegranate [28]. These differences likely reflect differences in methods, including extract concentration, extraction method and method of processing. Polyphenols in pomegranate inhibit the enzyme glucosyltransferase, reducing extracellular polysaccharide synthesis and plaque formation [29] and exhibit antimicrobial activity against *P. gingivalis*, *A. actinomycetemcomitans* and *F. nucleatum* [30]. The 0.05% concentration used may not be sufficient for maximum plaque control. Further studies should investigate optimal concentration and standardize formulations, given the differences in bioactive content depending on the pomegranate variety, source and extraction method. It's noteworthy that no side effects were observed in the group taking pomegranate extract. Chlorhexidine typically causes tooth discoloration, taste changes and mucosal irritation after two weeks [4], whereas herbal mouthwashes are well tolerated [31, 32]. This good safety profile may contribute to longer treatment durations. Our results have important clinical implications. For patients experiencing side effects from chlorhexidine or who prefer natural products, pomegranate extract is an effective alternative that has the potential to prevent gingivitis and is well tolerated, making it a promising option for periodontal disease prevention. The study's limitations include its small sample size (20 participants) and short duration (21 days), which hinder the generalizability and long-term interpretation of the results. Further research should include larger samples, longer follow-up and more outcomes

incorporating microbiological and inflammatory markers. Standardizing the pomegranate formulation will provide more consistent results and facilitate comparisons between studies.

Conclusion:

We show that mouthwash containing pomegranate peel extract (*Punica granatum*) has significant efficacy in reducing gingivitis exhibiting superior efficacy over a 0.2% chlorhexidine mouthwash. The combination of its potent anti-inflammatory properties, mild antimicrobial activity, safety profile, ease of preparation and excellent patient tolerance makes pomegranate extract mouthwash a promising natural alternative. However, when it comes to plaque control, CHX remains the gold standard. Therefore, further clinical trials with long-term follow-up are necessary to confirm the effectiveness of pomegranate peel mouthwash.

Advancement to Knowledge:

For patients experiencing side effects from chlorhexidine or who prefer natural products, pomegranate extract is an effective alternative that has the potential to prevent gingivitis and is well tolerated, making it a promising option for periodontal disease prevention. Pomegranate peel extract exhibits significant antibacterial activity against *S. mutans*, *P. gingivalis* and other oral tissue pathogens. Pomegranate extracts would exhibit anti-inflammatory activity at least as potent as chlorhexidine and may also have fewer side effects.

Acknowledgments:

The authors are very grateful to Devasthali Vidyapeeth College of Pharmacy, Haldwani for their valuable help in preparing mouthwash from pomegranate peel extract.

Funding:

Nil

Conflicts of interest: The authors declare no conflicts of interest.

Data availability statement: The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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