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## **Comparison and Evaluation of the Efficacy of Grape Seed Extract, Pomegranate Mouthwash, and Chlorhexidine Mouth Rinse in Treating Mild Gingivitis and Plaque in Children -Randomised Control Trial**

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### **Abstract**

**Objectives:** Maintaining optimal oral hygiene in children is essential for preventing periodontal diseases and ensuring long-term oral health. This randomized controlled trial (RCT) aimed to compare the efficacy of grape seed extract (GSE) mouthwash, pomegranate mouthwash, and chlorhexidine (CHX) mouth rinse in reducing plaque accumulation and gingivitis in children aged 6–10 years.

**Materials and Methods:** Thirty-three children meeting the inclusion criteria (Grade I and II Gingival Index [Loe & Silness, 1967] and Plaque Index [Silness & Loe, 1964], with at least one erupted first permanent molar) were randomly assigned to three groups: GSE mouthwash, pomegranate mouthwash, and CHX mouth rinse

(control). Plaque and gingival scores were recorded at baseline and at weekly intervals for three weeks. Statistical analysis was conducted using one-way ANOVA and post-hoc tests, with a significance level of  $p < 0.05$ .

**Results:** Pomegranate mouthwash demonstrated the greatest reduction in plaque accumulation, while both GSE and pomegranate significantly improved gingival health. Although CHX remains the gold standard, the plant-derived mouthwashes showed comparable efficacy with potential benefits for oral tissue health.

**Conclusion:** GSE and pomegranate mouthwashes may serve as effective natural alternatives for managing mild gingivitis and plaque in pediatric patients.

**Keywords:** Chlorhexidine, Gingivitis, Grape Seed Extract, Pediatric Oral Health, Plaque, Pomegranate Mouthwash

## Introduction

Prevention of oral diseases is very important to maintain good oral health condition; children usually neglect oral hygiene measures leading to dental diseases which subsequently affect their general health.<sup>1</sup> Dental plaque is a biofilm which is present on all tooth surfaces which initially serves a protective role by inhibiting the colonization of potentially harmful microbial species.<sup>2</sup> Despite this initial benefit, plaque can become detrimental when there is a sustained drop in its pH which promotes microbial imbalance and compromises oral health. This necessitates effective Plaque control to prevent the development of gingival inflammation and periodontal disease.

Achieving optimal oral hygiene remains a challenge for many individuals due to inconsistent oral care routines, inadequate brushing duration, and limited dexterity.<sup>5</sup> These limitations contribute significantly to the widespread occurrence of gingivitis. As a result, relying solely on mechanical plaque removal is often insufficient. To enhance oral hygiene outcomes the use of dentifrices and mouthwashes formulated with chemical or herbal agents are used. These adjunctive products can help reduce microbial load, control plaque accumulation, and improve overall periodontal health when integrated into daily oral care practices.

Chlorhexidine (CHX) has been the gold standard agent used for plaque and gingivitis control, but its displeasing taste, staining of teeth, dehydration and painful mucosa limits its long term usage.<sup>6</sup>

This has prompted recent research to focus on herbal mouthwashes, for better oral health maintenance, owing

to their wide range of biological and medicinal activities, ease of availability, higher safety margins, and lower cost.

Pomegranate (*Punica granatum*), a predominant member of the Punicaceae family, has proven to exhibit anti-inflammatory, antimutagenic and antibacterial effects. Therapeutically beneficial constituents of pomegranate include ellagic acid, ellagitannins, punicallagins, punic acid, flavonoids, anthocyanidins, antocyanins, and estrogenic flavones.<sup>5</sup> These contribute to antioxidant defense and suppress inflammatory response causing decrease in gingival inflammation.

Grape seed extract (GSE) contains high concentration of proanthocyanidin, it represents a variety of polymers of flavanol such as monomeric catechin and epicatechin, gallic acid and polyphenols<sup>6,7,8</sup>. Proanthocyanidin (condensed tannin) has been shown to strengthen collagen in tissues by enhancing collagen cross links. They promote collagen production which inhibit bacterial growth and support periodontal health. Based on the above findings, This study aimed to compare the efficacy of GSE mouthwash, pomegranate mouthwash, and CHX mouth rinse in reducing plaque and gingivitis in children<sup>9,10,11</sup>.

## Materials and Methods

### Study design, treatment and grouping

This study was a Randomized controlled trial which was conducted on 33 healthy children aged 6–10 years. Local factors that influence plaque deposition formed the basis of inclusion and exclusion criteria, including the presence of at least one fully erupted first permanent molar and mild to moderate gingivitis and not taking any current medication. Children with systemic diseases, allergies to herbal products, or those who had recently undergone

antibiotic or professional dental treatments were excluded from the study.

All patients and parents were informed about the study aims and the need of their cooperation to minimize the effect of confounding factors upon the treatment outcome. They were educated about the brushing method to be used (..) and was provided with soft bristled toothbrush (colgate). The patients received a coded bottle that contained the mouthwash. Patients were instructed to use their mouthwash after brushing twice daily and refrain from drinking ,eating, and mouth rinsing for 30minutes.

The diagnosis of gingivitis was based on clinical guidelines and included a detailed history, sign and symptoms and use of disclosing agent was used to disclose dental plaque. It was assessed using the Gingival Index (Loe & Silness, 1967). Additionally, in cases of gingivitis the plaque accumulation was graded on the extent and severity of bleeding on probing. using the Plaque Index (Silness & Loe, 1964), ensuring that participants presented with mild to moderate plaque deposits.

#### **Ethical clearance**

The study protocol was approved by the Institutional Ethical Committee, and informed consent was obtained from the parents or guardians of all participants. The trial was conducted in accordance with the principles outlined in the Declaration of Helsinki.

#### **Randomization and grouping**

The participants were randomly assigned to one of three groups using a computer-generated randomization sequence to minimize selection bias:

- **Group 1 (GSE Mouthwash Group):** Participants received grape seed extract (GSE) mouthwash.

- **Group 2 (Pomegranate Mouthwash Group):** Participants used pomegranate mouthwash.
- **Group 3 (Control Group):** Participants were provided with a commercially available 0.12% chlorhexidine (CHX) mouth rinse.

#### **Preparation of Herbal Mouthwash**

The herbal mouthwashes (GSE and pomegranate) were prepared under controlled laboratory conditions to maintain consistency in the concentration of active ingredients. The preparation followed standardized protocols to ensure safety and efficacy.

#### **Mouthwash Administration**

Participants were instructed to rinse with 10 mL of the assigned mouthwash twice daily after brushing their teeth. Compliance with the mouthwash regimen was monitored by parents or guardians, who maintained a log of mouthwash usage.

#### **Clinical evaluation**

Comparative evaluation were done in each group after evaluating the data for clinical parameters that were recorded at baseline and at 7,14 and 21 days at regular follow up.

- Plaque index
- Gingival index

At each follow up for each clinical parameter, the examinations were performed by 2 different evaluators to minimize assessment bias. The measurement was repeated by another assessor. Calibration of evaluators was carried out prior to the study to ensure consistency in data recording.

#### **Statistical Analysis**

The collected data were analyzed using SPSS software for windows (version 21). Descriptive statistics, including mean and standard deviation, were calculated for each group. One-way ANOVA was used to compare mean

differences within and between the groups, followed by post-hoc tests for multiple comparisons. A significance level of  $p < 0.05$  was considered statistically significant.

**Results**

A significant reduction in plaque scores was observed across all three groups over the three-week study period ( $p < 0.05$ ). Among the groups, pomegranate mouthwash demonstrated the highest reduction in plaque scores, followed by chlorhexidine (CHX) and grape seed extract (GSE) mouthwashes. Intragroup comparisons revealed statistically significant difference for each group whereas intergroup comparison showed no statistically significant difference at any specific time point, indicating that the

**Plaque Index**

Table 1: Comparison of mean Plaque Index (PI) scores at three visits among Chlorhexidine, Grape Seed, and Pomegranate groups

Group	PI_visit1	PI_visit2	PI_visit3	P value
Chlorhexidine	1.536 +/- .2767	1.182 +/- .1537	.945 +/- .2505	0.000
Grape	1.573 +/- .3259	1.291 +/- .1921	.991 +/- .1700	0.000
Pomegranate	1.480 +/- .2700	1.300 +/- .2108	1.000 +/- .1563	0.000
P value	0.769	0.276	0.793	

Table 1 shows the mean Plaque Index (PI) scores across three visits in the Chlorhexidine, Grape Seed, and Pomegranate groups. All groups demonstrated a statistically significant reduction in plaque scores over time ( $P = 0.000$ ), indicating effective plaque control within each intervention. However, intergroup comparisons at each visit revealed no statistically significant differences (Visit 1:  $P = 0.769$ ; Visit 2:  $P = 0.276$ ; Visit 3:  $P = 0.793$ ), suggesting that the three agents were comparable in efficacy.

The significant p-values (**0.000**) for each group indicate that the reduction in gingival scores over the three visits is statistically significant. This means that each

effectiveness of Chlorhexidine, GSE, and Pomegranate in reducing plaque was similar. Although pomegranate showed slightly better outcomes in other observations, statistically, it was not significantly superior to Chlorhexidine or GSE in reducing plaque scores.

In terms of gingival health, both GSE and pomegranate mouthwashes significantly reduced gingival inflammation and bleeding. Pomegranate mouthwash exhibited the most pronounced effect, while CHX also improved gingival health but did not demonstrate significantly superior outcomes compared to the natural alternatives.

mouthwash was effective in reducing gingival inflammation over time.

Intergroup comparisons revealed that pomegranate mouthwash was significantly more effective in plaque reduction than GSE ( $p < 0.05$ ). However, there was no significant difference between GSE and pomegranate in terms of reducing gingival inflammation ( $p > 0.05$ ).

These findings suggest that pomegranate mouthwash may serve as a promising natural alternative for managing plaque and gingivitis in children, offering comparable efficacy to CHX while promoting oral health through natural means.

**Gingival Index**

Table 2: Comparison of mean Gingival Index (GI) scores at three visits among Chlorhexidine, Grape Seed, and Pomegranate groups

Group	GI_visit1	GI_visit2	GI_visit3	P value
Chlorhexidine	1.827 +/- .3663	1.409 +/- .3618	.864 +/- .1804	0.000
Grape	1.400 +/- .3130	1.073 +/- .2102	.855 +/- .1809	0.000
Pomegranate	1.490 +/- .3414	1.200 +/- .2357	.890 +/- .1595	0.000
P value	0.016	0.027	0.891	

Table 2 presents the mean Gingival Index (GI) scores across three visits. All groups showed a significant reduction in gingival inflammation over time ( $P = 0.000$ ), confirming intra-group efficacy. Inter-group comparisons revealed significant differences at Visit 1 ( $P = 0.016$ ) and Visit 2 ( $P = 0.027$ ), with grape seed extract demonstrating lower baseline and mid-treatment scores. By Visit 3, however, no significant difference was observed among the groups ( $P = 0.891$ ), indicating comparable outcomes in gingival health.

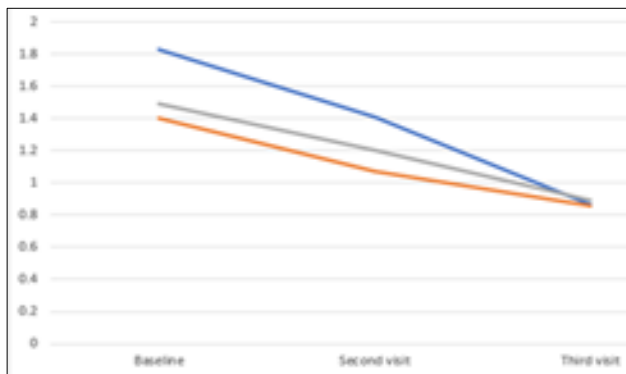


Figure 1: plaque index

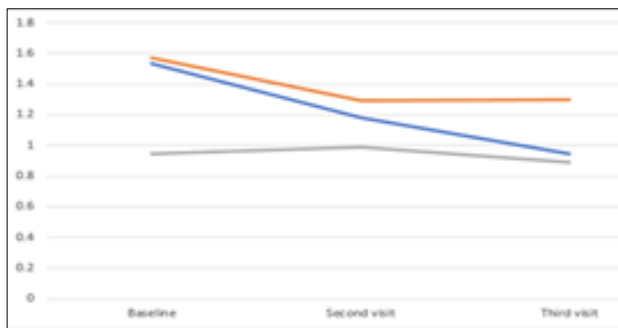


Figure 2: Gingival index

**Plaque Index- Figure 1:**

The graph depicting the Plaque Index shows a significant reduction in plaque scores over the three-week period across all three groups. Initially, all groups exhibited comparable plaque levels, but by the end of the study, the pomegranate mouthwash group demonstrated the most substantial decrease in plaque scores, followed by the CHX group and the GSE group. This indicates that pomegranate mouthwash was the most effective in reducing plaque accumulation among the three tested mouthwashes. The consistent decline in plaque scores over time suggests that regular use of pomegranate and CHX mouthwashes contributes to maintaining low plaque levels, while GSE mouthwash also shows a moderate yet significant reduction.

**Gingival Index-FIGURE 2-**The Gingival Index graph reveals a noticeable reduction in gingival inflammation and bleeding scores in all groups throughout the study duration. Both GSE and pomegranate mouthwashes significantly decreased gingival inflammation, with pomegranate showing the most pronounced effect. CHX also demonstrated improvement in gingival health, although it did not outperform the natural alternatives significantly. The similarity in the reduction of gingival scores between GSE and pomegranate indicates that both natural mouthwashes are effective in managing gingival

inflammation and bleeding, with pomegranate exhibiting a slightly superior outcome.

### Discussion

The results of this study highlight the potential of pomegranate-based mouthwashes and grape seed extract (GSE) as natural substitutes for chlorhexidine (CHX) in the treatment of moderate gingivitis and plaque buildup in paediatric patients. Children and teenagers are more prone to gingival inflammation, and epidemiological data suggests that gingivitis of some severity is nearly always present in this age range. Although periodontal disorders in children have historically received less attention due to the temporary nature of the primary dentition, early detection and treatment are still crucial. Regular periodontal examinations must be a part of paediatric dental appointments in order to guarantee prompt intervention and to support long-term oral health results.<sup>13</sup> Gingival infections can often be prevented with good oral hygiene and supportive products like mouth rinses. Although these chemotherapeutic agents are good at controlling plaque, they can have unintended side effects, including tooth discolouration, taste changes, gum peeling, and oral discomfort.<sup>14</sup> Pomegranate (*Punica granatum*) is one such fruit that has been shown to provide potent antibacterial and antioxidant properties without the typical negative effects. According to a research pomegranate juice and cold-pressed seed oil have antioxidant qualities that are stronger than red wine and comparable to those of green tea and butylated hydroxyanisole (BHA)<sup>15</sup> According to DiSilvestro et al. (2009), pomegranate components have qualities that may lower the risk of gingivitis and improve general dental health.

The quantity of plaque accumulation over a five-day period following the administration of pomegranate, chlorhexidine, and placebo mouthrinse was measured by Bhadbhade et al. (2011). The pomegranate group reduced as much plaque as the chlorhexidine rinse and had considerably less plaque buildup at the end of five days compared to the placebo group.<sup>16</sup> Research by Menezes et al. (2006) and Pai et al. (2010) showed that pomegranate fruit hydroalcoholic extract (HAE) has antibacterial properties against dental plaque and also inhibits the growth of *Staphylococcus aureus* and *Streptococcus pyogenes*.

Bioactive phenolic chemicals, which are abundant in the grape vine (*Vitis vinifera*), are primarily in charge of the plant's potent antibacterial and antioxidant qualities. Significant inhibitory effects have been demonstrated by these substances against oral pathogens, specifically *Streptococcus mutans* and other anaerobic bacteria linked to periodontal disorders.<sup>17</sup> Grape seed extract (GSE) may also be a promising remineralising agent for early enamel defects, according to in vitro research.<sup>18</sup> Smullens et al.'s study showed that polyphenol-rich grape extracts successfully prevent *S. mutans* from adhering to glass surfaces, suggesting that they may have a function in preventing the formation of biofilms.<sup>17</sup> Apart from its dental advantages, *Vitis vinifera* has been connected to more extensive medicinal uses, underscoring its worth as a flexible natural remedy for oral and systemic health.<sup>18</sup> chlorhexidine (CHX) has been the most extensively studied and is considered the gold standard for chemical plaque inhibition and gingivitis prevention. However, prolonged use of CHX has been associated with several local side effects, including brown staining of teeth and restorations, as well as altered taste sensation. As an alternative, the use of herbal mouth rinses has gained

popularity in recent years.<sup>20</sup> These natural formulations have demonstrated efficacy in plaque control while offering the advantage of minimal or no reported side effects, making them a favourable choice in modern dental practice, supported by the result of this study.

Numerous studies in the literature have explored the benefits of plant-based extracts used as mouthwashes in the management of periodontal conditions, often focusing on the effects of individual herbal formulations. However, there is a lack of comparative studies evaluating multiple herbal mouthwashes with chlorhexidine simultaneously, particularly in the context of their potential as antiplaque and antigingivitis agents in the pediatric population. The present study aims to address this gap by comparing two commonly used herbal extracts formulated as mouthwashes with chlorhexidine mouthwash with an emphasis on identifying an optimal concentration that ensures efficacy against dental plaque and gingivitis, maintains an acceptable taste profile for younger individuals, and minimizes potential side effects.

The comparable efficacy of these natural mouthwashes underscores the growing interest in plant-based therapeutics for oral health care. Their incorporation into daily oral hygiene routines could offer a safer and more sustainable approach to plaque and gingivitis management in children. Given their biocompatibility, minimal side effects, and potential long-term benefits, GSE and pomegranate mouthwashes may serve as effective adjuncts to conventional oral hygiene practices. Further clinical studies with larger sample sizes and extended follow-up periods are necessary to confirm their long-term efficacy and establish standardized formulations for optimal results.

## **Conclusion**

This study demonstrated that both GSE and pomegranate mouthwashes are effective in reducing plaque accumulation and improving gingival health in children. Pomegranate exhibited superior plaque control, while GSE and pomegranate significantly improved gingival health. Given their natural composition and additional oral health benefits, these mouthwashes may serve as promising alternatives to CHX in pediatric populations. Further research with larger sample sizes and longer follow-up periods is recommended to validate these findings.

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