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# Comparative Evaluation of Pomegranate Seed Extract and Magnetized Water as Mouth Rinses in Children: An in Vivo Study

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**KEYWORDS**

Pomegranate seed extract, Magnetized water, mouth rinse, antimicrobial efficacy, oral hygiene, children, in vivo study.

**ABSTRACT:**

**Introduction**: Maintaining oral hygiene is crucial in preventing dental caries and periodontal diseases, especially in children. However, traditional mouth rinses containing chemical agents may pose risks for young users. Natural alternatives like Pomegranate seed extract and Magnetized water have shown potential antimicrobial benefits. This study evaluates the efficacy of these two natural options as mouth rinses in children aged 6-12 years.

**Objectives**: To compare the antimicrobial efficacy of Pomegranate seed extract and Magnetized water as mouth rinses in children aged 6-12 years.

**Methods**: In this in vivo study, 60 children aged 6 to 12 years were randomly divided into three groups: Pomegranate seed extract rinse, Magnetized water rinse, and a Control group using saline. Participants used their assigned rinse twice daily for four weeks. The microbial load in their saliva was measured before and after the intervention, along with assessments of any side effects and oral comfort.

**Results**: Pomegranate seed extract and Magnetized water significantly reduced oral microbial load compared to the Control. No adverse effects were observed, and children using Pomegranate seed extract reported feeling fresher.

**Conclusions**: Pomegranate seed extract and Magnetized water are effective, natural mouth rinses that can enhance oral hygiene in children, offering a safe and well-tolerated alternative to chemical-based products. Further studies are recommended to investigate their long-term benefits and underlying mechanisms.

## 1. Introduction

Oral health plays a critical role in overall well-being, influencing physical health, self-esteem, and quality of life. Ensuring good oral hygiene is especially important in children, as it sets the stage for lifelong dental health. The oral cavity hosts a complex ecosystem of microorganisms, where some are beneficial and others can turn pathogenic if not properly managed. Effective oral hygiene practices are essential to maintain this balance and prevent oral diseases. Mouth rinses are a fundamental tool in oral health maintenance, as they are easily accessible and effective in reducing microbial load, preventing dental caries, and promoting overall oral hygiene across different age groups, particularly in children[1].

Children aged 6 to 12 years have unique oral health needs.[2] During this developmental phase, they are particularly vulnerable to dental caries, a common issue characterized by the demineralization of tooth enamel, largely due to acid-producing bacteria such as *Streptococcus mutans* and *Lactobacillus* species[3]. These bacteria colonize the tooth surface, produce acid from sucrose foods, and contribute to enamel demineralization. Preventing dental caries is essential, as untreated caries can lead to pain, infection, and even tooth loss, which negatively affects a child's overall health and development. Key strategies for preventing caries include minimizing tooth demineralization, modifying dietary habits, inhibiting or altering oral microbial growth, and enhancing salivary pH and buffering capacity[4].

Traditional mouth rinses, which often contain antimicrobial agents like Chlorhexidine, fluoride, or essential oils, are commonly used to combat dental caries and other oral health concerns[5-6]. Chlorhexidine is

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particularly flavoured for its broad-spectrum antimicrobial activity, but it also has drawbacks such as tooth staining, unpleasant taste, altered taste sensation, and increased calculus formation, which may reduce its acceptability, especially among children[7]. In recent years, there has been increasing interest in natural and alternative oral care solutions, which are being explored for their potential to provide similar or even superior benefits to traditional mouth rinses, with fewer side effects and greater acceptability[8]. Natural medicines have been utilized for thousands of years and continue to be an essential component of primary health care, particularly in underdeveloped and developing countries[9]. Various herbal products, such as Propolis, *Emblica officinalis* (Amla), *Ocimum sanctum* L. (Tulsi), and *Azadirachta indica* (Neem), have shown significant efficacy in reducing gingival and periodontal inflammation and inhibiting the growth of *S. mutans* and *Lactobacillus*[9].

Pomegranate (*Punica granatum*), known for its rich phytochemical content and potential health benefits, contains bioactive compounds such as ellagic acid, anthocyanins, and tannins, which exhibit strong antioxidant and antimicrobial properties. Pomegranate has been cultivated since prehistoric times in the Mediterranean region and is highly valued in Ayurvedic medicine for its wide range of therapeutic properties, often referred to as a "pharmacy unto itself"[10-11]. Research indicates that pomegranate extract can inhibit the growth of oral pathogens, reduce inflammation, and support overall oral health. Despite these promising findings, its use as a mouth rinse in pediatric oral care has not been widely studied. This study aims to address this gap by evaluating the efficacy of pomegranate seed extract as a mouth rinse for children.

Magnetized water, also referred to as structured water, is an emerging concept proposed as an alternative to conventional mouth rinses. This type of water is produced by exposing regular water to strong magnetic fields, which is believed to alter its physical and chemical properties, potentially enhancing its antimicrobial effects and bioavailability[12-15]. The appeal of magnetized water lies in its natural and non-invasive approach, offering a more child-friendly alternative to traditional mouth rinses, which may have strong flavours or sensations that can be unpleasant for children. However, the scientific evidence supporting its effectiveness in oral care remains limited, highlighting the need for further research.

## 2. Objectives

This study aims to thoroughly examine and compare the antimicrobial efficacy of Pomegranate seed extract and Magnetized water as mouth rinses in children aged 6 to 12 years in an in vivo setting. By exploring these natural and alternative solutions, the study seeks to identify effective, safe, and acceptable options for enhancing pediatric oral health.

## 3. Methods

Materials and Instruments

Preparation of Pomegranate Extract:

Pomegranate juice was freshly extracted from a single pomegranate fruit for each preparation, ensuring consistency in the bioactive components of the mouthrinse.


Fig. 1 Pomegranate extracts.

**Preparation of Magnetized Water:**

Water was purified through reverse osmosis and stored in glass bottles. These bottles were then exposed to a magnetic field with a strength of 1000 Gauss for 24

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hours. This process was hypothesized to modify the water's physical and chemical properties, potentially enhancing its antimicrobial effects.

Fig. 2 Magnetised water.

Fig. 3 Magnetised water with bubbles formation.

**Study Design**

The research was conducted as an in vivo randomized controlled trial involving children aged 6 to 12 years. Participants were randomly assigned to three groups: Group A (Pomegranate seed extract mouthrinse), Group B (Magnetized water mouthrinse), and Group C (Control group using distilled water). Each group used the assigned mouthrinse twice daily for 4 weeks.

**Study Setting**

The study took place in Mumbai City, Maharashtra, for 2 months. A total of 42 children visiting the department were recruited for participation. The sample size was calculated using the following parameters:

- *Population Size (N):* 42.
- *Hypothesized % Frequency (p):* 7.33% ± 5
- *Confidence Limit (d):* 5%
- *Design Effect (DEFF):* 1

The calculated sample size was 42 participants. These 42 participants were selected and divided into three groups, with each group consisting of 14 participants.

**Convenience Sampling**

Participants were selected through convenience sampling using a randomized computerized number table to ensure unbiased group assignment.

**Method of Selection**

**Inclusion Criteria:**

- Healthy children aged 6 to 12 years.
- dmft/DMFT index score equal to or less than 4.
- Systemically healthy children in mixed dentition with no history of antibiotics or mouthwash use in the past month.

**Exclusion Criteria:**

- Children with fixed or removable orthodontic appliances or prostheses.
- Children with abscesses, draining sinuses, cellulitis, or conditions requiring emergency medical treatment.
- Children with severe malalignment of the teeth.

**Withdrawal Criteria:**

- Participants who chose to withdraw from the study at any time.

**Method of Data Collection**

**Saliva Collection:**

Unstimulated saliva samples were collected at two time points: immediately after rinsing and one hour after rinsing. This provided data on both immediate and short-term effects of the mouthrinses.

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**Microbial Assessment:**

- *S. mutans Assessment:* Saliva samples were cultured on Mutans Sanguis agar to quantify Streptococcus mutans.
- *Lactobacilli Assessment:* Saliva samples were cultured on Rogosa agar to quantify Lactobacillus species.

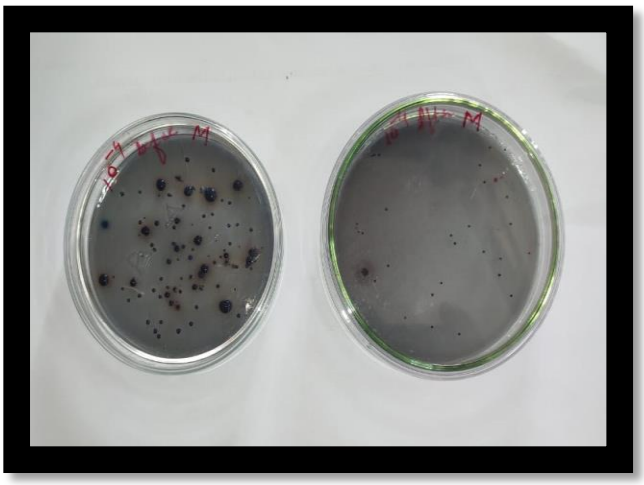

Fig.4 Baseline culture for Mutans Sanguis agar

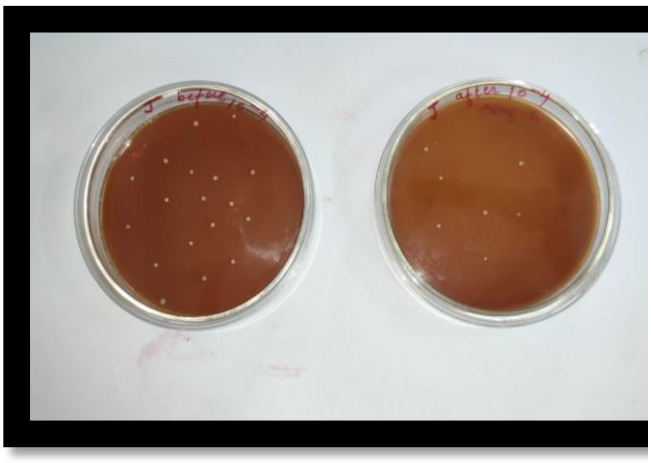

Fig.5 Baseline culture for Ragosa agar

**Mouthrinse Administration:**

Participants used 5 ml of the assigned mouthrinse, and were instructed to rinse for one minute. The mouthrinses were stored in opaque bottles to avoid any bias.

**Examination Procedure**

**Colony Counting:**

Bacterial colonies on agar plates were manually counted to determine bacterial load.

**Bacterial Smear Staining:**

Bacterial smears were prepared and stained with Gram's stain to identify bacterial types and confirm morphology.

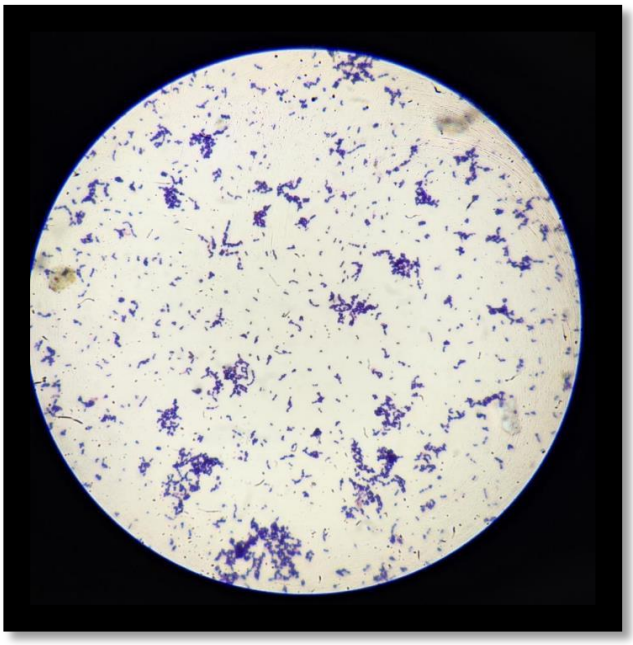

Fig.6 Gram staining of Streptococcus Mutans.

**Analysis Plan and Methods**

**Data Entry and Processing:**

Data were entered into Microsoft Excel (version 13) for organization and initial processing.

**Statistical Analysis:**

- *Descriptive Statistics:* Mean and standard deviation of bacterial counts and other relevant variables were calculated.
- *Inferential Statistics:* The Chi-Square test was used to compare categorical variables between groups. Normality of data was assessed using the Kolmogorov-Smirnov Test. Depending on normality, either parametric or non-parametric tests were applied.

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- *Significance Level:* Analysis was conducted at a 95% confidence interval, with p-values less than 0.05 considered statistically significant.
- *ANOVA:* Analysis of Variance was used for inter-group comparison. Post-hoc tests were conducted if ANOVA showed significant differences.

**Further Procedures**

**Follow-Up Analysis:**

Significant findings were further analyzed to explore differences in more detail and to examine trends over time within each group.

**Reporting:**

The findings were compiled into a comprehensive report, including statistical tables, graphs, and interpretations of results.

**Ethical Considerations:**

Informed consent was obtained from parents or guardians of participating children. Ethical guidelines were followed throughout the study, and any reported adverse effects were promptly addressed. Participants had the right to withdraw from the study at any time.

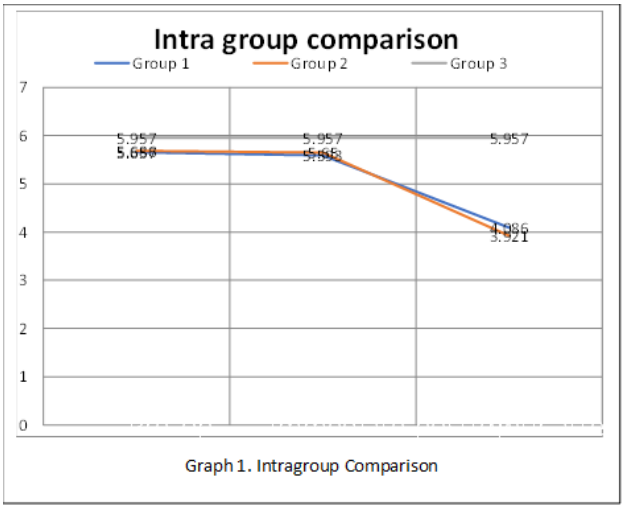
This methodology ensured a thorough and scientifically rigorous investigation into the comparative efficacy of pomegranate seed extract and magnetized water as mouthrinses for children aged 6 to 12 years.

## 4. Results

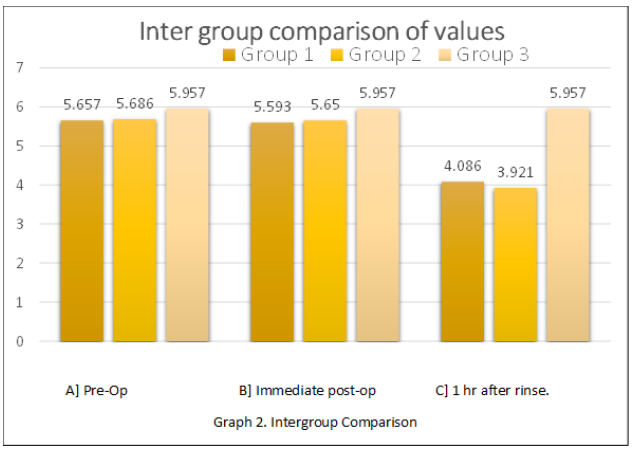
**Intragroup Comparison**

**Pomegranate Seed Extract Mouthrinse (Group A):** The use of pomegranate seed extract mouthrinse led to a statistically significant reduction in the salivary S. Mutans count. The initial mean count of S. Mutans was 5.957, which decreased to 5.593 immediately after rinsing and further declined to 4.086 one hour post-rinse. This reduction was statistically significant, with a p-value of 0.001.

**Magnetized Water Mouthrinse (Group B):** The magnetized water mouthrinse also showed a significant reduction in S. Mutans counts. The initial mean count was 5.686, which dropped to 5.593 immediately after rinsing and further decreased to 3.921 one hour post-rinse. This reduction was statistically significant, with a p-value of 0.001.




Graph 1. Intragroup Comparison




Graph 2. Intergroup Comparison

**Control Group (Group C):** The control group, which used distilled water, did not exhibit any significant change in S. Mutans counts. The mean counts remained consistent at 5.957 across all time points, indicating no antimicrobial activity.

**Intergroup Comparison**

**Comparison Between Groups:**

- **Pre-Op:**

  The baseline S. Mutans counts were similar across all groups.

- **Immediate Post-Op:**

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Both Group A (Pomegranate seed extract) and Group B (Magnetized water) showed a statistically significant decrease in S. Mutans counts compared to the control group (Group C). However, no statistically significant difference was observed between Group A and Group B.

- **1 Hour After Rinse:**

  The significant reduction in S. Mutans counts persisted in both Group A and Group B compared to the control group, but no significant difference was found between the two experimental groups.

**Lactobacilli Counts:**

- The Lactobacilli counts did not show statistically significant differences between the groups, indicating that neither Pomegranate seed extract nor Magnetized water had a differential effect on Lactobacilli.

## 5. Discussion

Lorem The findings of our study align with previous research on the antimicrobial effects of pomegranate and magnetized water mouthrinses, further emphasizing their potential as effective alternatives to conventional mouthwashes. Unlike prior studies that typically compared these alternatives to established mouthwashes, our study uniquely examined the direct comparison between Pomegranate seed extract and Magnetized water mouthrinses.[16-17]

Bhadbhade (2011) [18] found no statistically significant difference in terms of plaque index (PI) when comparing a pomegranate rinse with a standard mouthwash, which supports our findings of the pomegranate mouthrinse's efficacy in reducing bacterial counts. Singla et al. (2018)[19] reported significant antimicrobial effects using a 15 ml dose of pomegranate extract twice a day. Our methodology, which involved freshly prepared pomegranate extract, also demonstrated a significant reduction in S. Mutans counts, reinforcing the effectiveness of the pomegranate extract. Similarly, Goyal et al. (2017)[20] observed a significant reduction in S. Mutans count in plaque with the use of pomegranate mouthrinse, paralleling our results of substantial reductions in salivary S. Mutans, thereby confirming the antibacterial properties of pomegranate extract. Umar et al. (2016)[21] found an increase in salivary pH after using pomegranate mouthrinse for 10 minutes. Although our study did not measure salivary pH, the observed reduction in bacterial counts suggests an improvement in the oral environment, which could potentially influence pH levels.

Nezam S et al. (2022)[22] found that both magnetized water and standard mouthwash were effective in managing periodontal and gingival infections, a finding consistent with our study which showed significant reductions in S. Mutans counts with Magnetized water. This underscores the potential of magnetized water as an antimicrobial mouthrinse. While previous studies primarily focused on the antimicrobial properties of magnetized water, our study adds to the literature by directly comparing it to Pomegranate extract, demonstrating that both have similar effectiveness in reducing salivary microbial counts.

One notable challenge in our study and previous research is the labor-intensive preparation process for pomegranate mouthrinse, which needs to be freshly prepared to maintain its bioactive components. This can be a limiting factor for its widespread use. In contrast, preparing magnetized water involves purifying water through reverse osmosis and then exposing it to a magnetic field, which is less labor-intensive compared to preparing pomegranate extract.

Pomegranate peel extract contains a rich blend of bioactive compounds, including punicalagin, ellagic acid, tannins, and flavonoids, which contribute to its antimicrobial, anti-inflammatory, and antioxidant properties. Microorganisms present on the tongue are responsible for releasing sulfur-containing compounds that cause halitosis. Saliva plays an important role in maintaining good oral health, and a reduced saliva flow during sleep favors anaerobic bacterial breakdown, giving rise to bad odor. This results from decreased salivary flow and reduced activity of tongue and cheek muscles during sleep, promoting bacterial growth. Plaque is a known initiating factor in the development of gingivitis, making plaque control essential for good oral hygiene. Salivary pH also plays a crucial role in halitosis formation. Antibacterial components such as cetylpyridinium chloride, triclosan, essential oils, chlorine dioxide, zinc salts, benzalkonium chloride,

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hydrogen peroxide, and sodium bicarbonate are used to maintain salivary pH. Therefore, new antimicrobial, anti-inflammatory, and antioxidant agents of plant origin, which are safe, preventive, and economical, are needed.

Our study highlights the significant antimicrobial properties of pomegranate peel extract. Pomegranate peel contains punicalagin, flavones, and tannins such as punicalin and punicafolin, which inhibit human salivary alpha-amylase. This enzyme catalyzes the hydrolysis of starch to oligosaccharides and binds to viridans streptococci and enamel, providing a substrate for cariogenic microorganisms. Pomegranate peel extract combats dental plaque and reduces calculus formation by discouraging the action of microorganisms that initiate plaque formation. Various in vitro studies have supported the antibacterial efficacy of pomegranate peel extract. For instance, Lee et al. (2005)[23] stated that pomegranate extracts inhibit sucrose-digesting enzymes and plaque-forming organisms through competitive and noncompetitive inhibition. Polyphenolic flavonoids in pomegranate are effective in maintaining good oral health and preventing gingivitis Hajifattahi et al. (2016) [24]evaluated the hydroalcoholic extract of pomegranate on various oral microorganisms, finding significant antibacterial effects, especially on S. mutans. Vasconcelos et al.[25] found that pomegranate phytotherapeutic gel had greater efficiency in inhibiting microbial adherence than miconazole.

Magnetized water, prepared by exposing purified water to a magnetic field, has been proposed to exhibit unique physical and chemical properties that enhance its antimicrobial effects. The magnetic treatment is believed to alter the hydrogen bonding structure of water, increasing its solubility and reactivity. This process potentially enhances the water's ability to disrupt bacterial cell walls and inhibit microbial growth. In our study, magnetized water showed significant reductions in S. Mutans counts, demonstrating its effectiveness as an antimicrobial agent. Previous studies, such as those by Nezam S et al. (2022)[22], have also reported similar findings, highlighting the efficacy of magnetized water in managing periodontal and gingival infections.

In our study, aqueous pomegranate peel extract increased salivary pH after 10 and 30 minutes of use, compared to a standard mouthwash, indicating its antibacterial properties. This suggests that pomegranate peel extract can replace synthetic mouthwashes to avoid side effects. Ahuja et al. [26] compared pomegranate and standard mouthwashes, concluding that pomegranate mouthwash is favorable in improving gingival status due to its styptic action and adequate plaque reduction. Menezes et al.[27] observed more significant plaque depletion with pomegranate (84%) compared to a standard mouthwash (79%) after 1 minute of rinsing. Abdollahzadeh et al.[28] conducted an in vivo study showing that pomegranate methanolic extract controls common oral pathogens responsible for caries, stomatitis, and periodontal diseases. The antibacterial activity of pomegranate may be related to its polyphenol structures, which can affect bacterial cell walls, inhibit enzymes, interact with proteins, and disturb microorganism coaggregation.

An Ohio State study reported that rinsing with pomegranate solution reduced saliva total protein content, which is higher in people with gingivitis and may correlate with plaque-forming bacterial content. Umar et al.[29] showed that pomegranate peel extract mouth rinse significantly lowered the salivary count of S. mutans compared to a standard mouthwash, and increased salivary pH within 10 minutes, demonstrating its potential as an anticariogenic agent. Our study produced similar results, showing a significant ($P < 0.001$) change in salivary pH scores at different time intervals, indicating the anticariogenic efficacy of pomegranate peel extract.

Pomegranate also lowers saliva activities of alpha-glucosidase, an enzyme that breaks down sucrose, while increasing activities of ceruloplasmin, an antioxidant enzyme, suggesting its anticariogenic effects. The present study showed a sudden increase in salivary pH after using pomegranate, indicating its potential to prevent dental caries. However, a limitation of our study was the use of pomegranate peel extract in a single concentration. Future investigations should determine the appropriate concentration of pomegranate peel extract for regular use as a mouthwash.[30]

The aqueous extract of pomegranate peel was as effective as a stand Future studies should focus on increasing the substantivity of herbal mouthwashes to provide a cost-effective, antimicrobial, and preventive alternative to commercially available mouthwashes. Both pomegranate seed extract and magnetized water are promising anti-cariogenic agents, as demonstrated by

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their significant reductions in salivary microbial counts in our study. Further research should explore the long-term benefits and broader antimicrobial spectrum of these mouthrinses to fully understand their potential in oral health care.

**Conclusion**

The findings from our study indicate that both pomegranate seed extract and magnetized water mouthrinses are highly effective in immediately reducing salivary microbial counts in children aged 6 to 12 years. Despite differences in preparation methods and bioactive components, both treatments exhibited comparable antimicrobial activity, particularly against Streptococcus mutans, a key contributor to dental caries. This suggests that both Pomegranate seed extract and Magnetized water have significant potential as natural, effective mouthrinses for maintaining oral hygiene and preventing dental caries.

Our results highlight the advantages of using these natural alternatives to conventional mouthwashes, offering a safer and potentially more cost-effective solution without the associated side effects of synthetic products. Future research should focus on exploring the long-term benefits and broader antimicrobial spectrum of these mouthrinses to fully understand their potential in oral health care. Additionally, further studies are needed to optimize the concentration and formulation of these mouthrinses for regular use, as well as to investigate their effects on a wider range of oral microorganisms and in different population groups. Both pomegranate seed extract and magnetized water mouthrinses show great promise as anti-cariogenic agents, with the potential to become mainstream alternatives to conventional mouthwashes. Their use could significantly enhance oral health care practices by providing effective, natural solutions for reducing bacterial load and maintaining oral hygiene.

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