

EFFECT OF MISWAK COCONUT OIL AND MOUTHWASH ON PLAQUE PROUCING BACTERIA

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ABSTRACT

Background: Dental plaque is a complex microbial biofilm associated with the development of dental caries and periodontal diseases. It is primarily formed by bacteria such as *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, and *Lactobacillus* species. These microorganisms metabolize dietary carbohydrates to produce acids that demineralize tooth enamel. Increasing concerns regarding the side effects of chemical oral hygiene products have led to growing interest in natural alternatives such as coconut oil and miswak for controlling plaque-forming bacteria.

Objective: To evaluate the effect of coconut oil, miswak, and mouthwash on plaque-producing bacteria and to compare their effectiveness in reducing bacterial growth.

Methods: A randomized experimental study was conducted involving three participants allocated to coconut oil, miswak, and chlorhexidine mouthwash groups. Oral samples were collected at baseline, day 7, and day 15. Coconut oil pulling was performed using 10 mL for 10 minutes daily, while miswak and mouthwash were used for one minute daily. Samples were cultured on appropriate media, and bacterial identification was confirmed using microscopy and biochemical tests. Bacterial load was quantified using colony-forming units per milliliter (CFU/mL). Data were analyzed using SPSS, and changes over time were assessed.

Results: Baseline bacterial counts were high across all groups, standardized at 3,000,000 CFU/mL. In the coconut oil group, bacterial load decreased to 490,000 CFU/mL on day 7 and further to 130,000 CFU/mL on day 15. In the miswak group, counts reduced to 2,230,000 CFU/mL on day 7 and 1,200,000 CFU/mL on day 15. The mouthwash group showed a reduction to 2,730,000 CFU/mL on day 7 but returned to baseline levels (3,000,000 CFU/mL) by day 15.

Conclusion: Coconut oil demonstrated the most effective and sustained antibacterial activity against plaque-producing bacteria, followed by miswak, while mouthwash showed limited long-term effectiveness. Natural oral hygiene methods may serve as effective alternatives for controlling oral bacterial load.

Keywords: Chlorhexidine, Coconut Oil, Dental Plaque, Miswak, Oil Pulling, Oral Hygiene, *Streptococcus mutans*

INTRODUCTION

Oral health is a fundamental component of overall well-being, yet it continues to be compromised globally by preventable conditions such as dental caries and periodontal disease. Central to these conditions is dental plaque, a complex biofilm that harbors diverse microbial communities capable of disrupting the balance of the oral environment (1,2). Among the most significant contributors to plaque-related pathology are acidogenic and aciduric bacteria, particularly those responsible for enamel demineralization and the progression of tooth decay. These microorganisms thrive within the oral biofilm, where their metabolic activity promotes sustained acidic conditions that ultimately damage both hard and soft oral tissues (3). In addition to dental caries, shifts in the oral microbiome can lead to inflammatory conditions such as gingivitis and periodontitis, further emphasizing the need for effective strategies to control microbial growth while maintaining microbial balance. Recent advances in microbiological research have reshaped the understanding of the oral ecosystem, highlighting its complexity and the symbiotic relationship between host and microorganisms (4). Rather than viewing all oral bacteria as harmful, contemporary perspectives recognize that many microbial species contribute positively to oral health by preventing colonization of pathogenic organisms. This paradigm shift underscores the limitations of approaches that rely solely on broad-spectrum antimicrobial elimination and instead supports more targeted and balanced interventions. Consequently, there is growing interest in alternative and adjunctive methods of plaque control that not only reduce pathogenic bacteria but also preserve the beneficial components of the oral microbiome (5).

Traditional oral hygiene practices, including the use of chewing sticks such as miswak, have been employed for centuries across various cultures due to their accessibility, affordability, and perceived therapeutic benefits. Miswak, derived from *Salvadora persica*, contains biologically active compounds with antimicrobial, anti-inflammatory, and plaque-inhibiting properties. These characteristics have been associated with improvements in gingival health and reductions in bacterial load, making it a valuable natural alternative or adjunct to conventional oral hygiene tools (6). Similarly, oil pulling, particularly with coconut oil, has gained attention as a complementary practice rooted in traditional medicine. The antimicrobial potential of coconut oil is largely attributed to its high content of medium-chain fatty acids, especially lauric acid, which can disrupt bacterial cell membranes and inhibit microbial adhesion (7). Evidence suggests that such practices may contribute to reductions in plaque accumulation and oral bacterial counts, although findings remain variable. In contrast, chemical agents such as antimicrobial mouthwashes continue to represent a cornerstone of modern oral hygiene. These formulations, often containing compounds like chlorhexidine or essential oils, are effective in reducing plaque formation and gingival inflammation through their broad-spectrum antimicrobial activity. However, their prolonged use has been associated with undesirable side effects, including tooth staining, taste alteration, and potential disruption of the natural oral microbiota (8). These limitations have prompted increasing interest in natural and holistic alternatives that may offer comparable benefits with fewer adverse effects.

Despite the individual effectiveness of miswak, coconut oil, and conventional mouthwashes, there remains a notable lack of direct comparative research evaluating their relative impact on plaque-producing bacteria (9). This gap highlights the need for studies that assess not only their antimicrobial efficacy but also their potential role in promoting a balanced oral microbiome. Understanding these effects is essential for developing evidence-based, accessible, and culturally relevant oral hygiene strategies. The present study is therefore designed to evaluate the effect of miswak, coconut oil, and mouthwash on plaque-producing bacteria and to compare their effectiveness in reducing bacterial growth. By addressing this gap, the study aims to contribute to the development of optimized oral care practices that integrate both traditional and modern approaches.

METHODS

This study was conducted as a randomized controlled trial to evaluate the effects of miswak, coconut oil, and chemical mouthwash on plaque-producing oral bacteria. Random allocation of participants into three parallel groups was performed to minimize selection bias and ensure comparability. Baseline dental plaque samples were collected prior to the initiation of interventions (T₀), followed by subsequent sampling after 7 days (T₁) and 15 days (T₂) to assess temporal changes in bacterial load. The control framework was structured such that each group followed a distinct oral hygiene intervention: one group used coconut oil, another used miswak, and the third used a conventional antimicrobial mouthwash (10,11). This design enabled an objective comparison of antibacterial efficacy across the interventions. The study was carried out at the Microbiology Laboratory of the Medical Laboratory Technology Department, Riphah International University, Faisalabad. The laboratory was equipped with standard and advanced microbiological facilities, including media preparation units, sterilization systems, incubators, and diagnostic tools necessary for microbial identification and antimicrobial analysis. All procedures were conducted under controlled laboratory conditions in accordance with established standard operating protocols, and strict biosafety measures were observed throughout sample handling and processing to ensure the reliability and validity of the findings.

The study population comprised individuals who met predefined eligibility criteria and consented to participate. Participants were randomly assigned to one of three intervention groups: coconut oil, miswak, or mouthwash. Inclusion criteria required participants to be in good general and oral health, with no clinical signs of active oral disease. Individuals with ongoing oral infections, recent dental procedures, or a history of recent antibiotic use were excluded to avoid confounding effects on the oral microbiota (12,13). Written informed consent was obtained from all participants prior to enrollment. Ethical approval for the study was obtained from the

institutional ethical review committee of Riphah International University (approval reference number not specified), and the study adhered to ethical standards concerning human subject research. Plaque samples were collected using sterile oral swabs from the same anatomical site—the central incisor tooth of each participant—to maintain consistency. Sampling was performed under aseptic conditions by trained personnel. Each sample was collected approximately 20 minutes after the use of the assigned oral hygiene intervention to standardize microbial activity levels across participants. The swabbing procedure involved gentle circular motions (approximately ten rotations) over the tooth surface for 10–15 seconds. All samples were properly labeled and transported promptly for laboratory analysis.

Upon collection, each sample was divided into two portions to allow parallel microbiological evaluation (14). One portion was used for the isolation and identification of bacterial species. Samples were inoculated onto blood agar plates and incubated under appropriate conditions to promote bacterial growth (15). Colony morphology and hemolytic patterns were initially assessed, followed by Gram staining and biochemical testing, including catalase testing. Optochin sensitivity testing was performed when necessary to aid in species differentiation. All procedures were conducted according to standard microbiological laboratory guidelines. The second portion of each sample was used for quantitative microbiological analysis. Samples were cultured on nutrient agar, and colony-forming units per milliliter (CFU/mL) were calculated using standard plate count methods. Only countable plates were selected to ensure accuracy. The CFU/mL was determined by multiplying the number of colonies by the appropriate dilution factor. This quantitative approach provided a reliable measure of bacterial load and enabled comparison across different time points and intervention groups (16). Statistical analysis was performed using SPSS software to evaluate differences in bacterial counts within and between groups. The Friedman test, a non-parametric statistical test, was applied to assess changes in CFU/mL over time within each group. A p-value of less than 0.05 was considered statistically significant. The analysis allowed for an objective assessment of the antimicrobial efficacy of miswak, coconut oil, and mouthwash interventions.

RESULTS

The oral isolate obtained from plaque samples was identified as *Streptococcus mutans* based on standard microbiological and biochemical characteristics. Growth on blood agar demonstrated a gamma-hemolytic, non-hemolytic pattern. The isolate exhibited resistance to optochin, effectively excluding optochin-sensitive streptococci. Gram staining revealed Gram-positive cocci arranged predominantly in chains, while the catalase test produced a negative result. Additionally, the Voges–Proskauer test was positive, further supporting the identification of the organism as *Streptococcus mutans*. At baseline, all groups demonstrated heavy bacterial growth, recorded as too numerous to count and standardized to 300 colonies, corresponding to 3,000,000 CFU/mL. Over the study period, distinct patterns of bacterial reduction were observed across the three intervention groups. In the coconut oil group, a marked and progressive reduction in bacterial load was observed. Colony counts decreased from 300 (3,000,000 CFU/mL) at baseline to 49 (490,000 CFU/mL) on day 7, and further declined to 13 (130,000 CFU/mL) by day 15. This represented an absolute reduction of 2,870,000 CFU/mL and an overall decrease of 95.7%. The reduction was rapid, with an initial decline of 83.7% by day 7, followed by continued reduction thereafter. In the miswak group, a moderate yet consistent decline in bacterial load was recorded. Colony counts reduced from 300 (3,000,000 CFU/mL) at baseline to 223 (2,230,000 CFU/mL) on day 7, and further to 120 (1,200,000 CFU/mL) by day 15. This corresponded to an absolute reduction of 1,800,000 CFU/mL and an overall decrease of 60.0%. The reduction pattern indicated a gradual and sustained antibacterial effect over time.

In contrast, the mouthwash group showed only a transient reduction in bacterial load. Colony counts decreased slightly from 300 (3,000,000 CFU/mL) at baseline to 273 (2,730,000 CFU/mL) on day 7, reflecting a 9.0% reduction. However, by day 15, the bacterial count returned to baseline levels of 300 (3,000,000 CFU/mL), indicating no sustained antibacterial effect over the study period. Within-group analysis using the Friedman test demonstrated a statistically significant reduction in bacterial counts over time in both the coconut oil group ($\chi^2 = 8$, $p = 0.018$) and the miswak group ($\chi^2 = 6$, $p = 0.049$). In contrast, the mouthwash group did not show a statistically significant change ($\chi^2 = 1$, $p = 0.607$). For comparative evaluation across interventions, the percentage reduction in bacterial load from baseline to day 15 was analyzed. Coconut oil demonstrated the highest reduction (95.7%), followed by miswak (60.0%), while mouthwash showed no net reduction (0%). The magnitude of reduction indicated that coconut oil achieved approximately 35.7% greater reduction than miswak and 95.7% greater reduction than mouthwash. The temporal pattern of reduction further highlighted differences between interventions. Coconut oil exhibited a rapid and sustained antibacterial effect, miswak showed a slower but consistent decline, whereas mouthwash demonstrated only a short-term effect without long-term benefit.

Table 1. Bacterial Load (CFU/mL) Over Time Across Study Groups

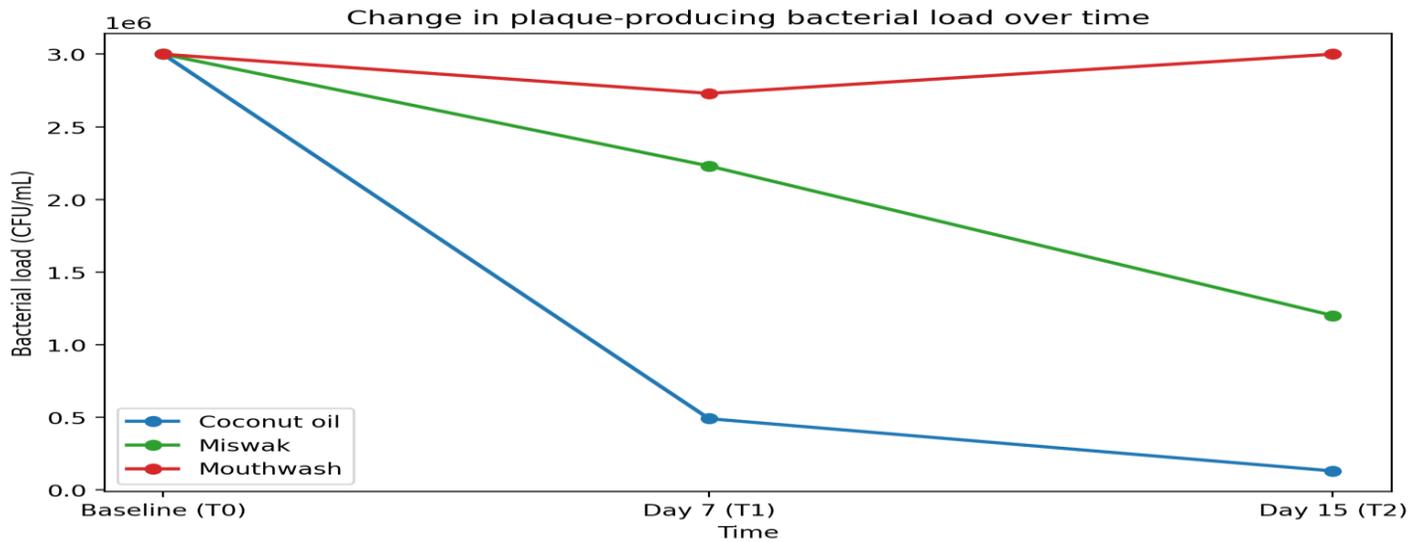
Time Point	Coconut Oil (CFU/mL)	Miswak (CFU/mL)	Mouthwash (CFU/mL)
Baseline (T0)	3,000,000	3,000,000	3,000,000
Day 7 (T1)	490,000	2,230,000	2,730,000
Day 15 (T2)	130,000	1,200,000	3,000,000

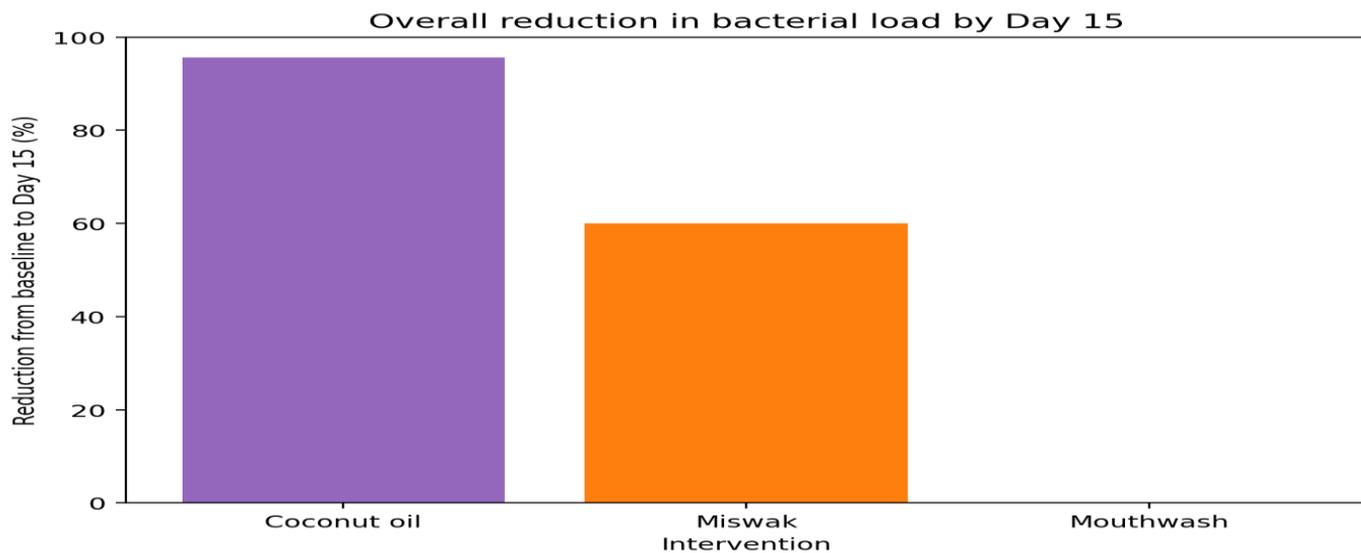
Table 2. Percentage Reduction in Bacterial Load from Baseline

Group	Day 7 Reduction (%)	Day 15 Reduction (%)	Overall Trend
Coconut Oil	83.7%	95.7%	Strong and sustained reduction
Miswak	25.7%	60.0%	Moderate and consistent reduction
Mouthwash	9.0%	0%	No sustained reduction

Table 3. Comparative Analysis of Effectiveness Among Group

GROUP	Friedman X2 (df=2)	p-value	Interpretation
Miswak	6	0.049	Significant reduction over time
Coconut oil	8	0.018	Highly significant reduction over time
Mouthwash	1	0.607	No significant changes





DISCUSSION

The present study evaluated the comparative effectiveness of coconut oil, miswak, and mouthwash in reducing plaque-producing bacteria, specifically *Streptococcus mutans*. The findings demonstrated a clear reduction in bacterial load in both the coconut oil and miswak groups over time, whereas the mouthwash group showed only a transient reduction with no sustained effect (17). These observations suggest that natural oral hygiene approaches may offer measurable antibacterial benefits under the conditions of this study. The most notable finding was the substantial reduction in bacterial count observed in the coconut oil group, which showed a rapid and sustained decline over the 15-day period. This supports the concept that oil pulling may contribute to oral hygiene through both mechanical and biochemical mechanisms (18). It has been proposed in previous studies that the process of oil pulling facilitates emulsification and saponification, which enhances the removal of bacteria from the oral cavity. Additionally, the presence of medium-chain fatty acids, particularly lauric acid, has been associated with antimicrobial activity through disruption of bacterial cell membranes and inhibition of adhesion. These mechanisms may explain the pronounced reduction in *S. mutans* observed in this study (19,20). However, some earlier investigations have reported that while oil pulling reduces overall bacterial load, it may not consistently outperform standard antimicrobial agents, indicating that its efficacy may depend on duration, frequency, and methodology of use.

The miswak group also demonstrated a consistent reduction in bacterial load, although the magnitude of reduction was less pronounced compared to coconut oil. This finding aligns with existing literature suggesting that miswak possesses both mechanical and chemical properties that contribute to plaque control. The fibrous structure of the stick allows for mechanical removal of plaque, while its bioactive constituents, such as benzyl isothiocyanate and flavonoids, exhibit antimicrobial effects (21,22). Previous studies have shown that regular use of miswak can significantly reduce plaque accumulation and improve gingival health, in some cases showing comparable effectiveness to conventional toothbrushing. The gradual decline observed in this study may reflect both its antimicrobial action and the learning curve associated with its use, as improper technique may limit its effectiveness. In contrast, the mouthwash group did not demonstrate a sustained reduction in bacterial load, despite a slight decrease observed at day 7. This finding differs from a substantial body of literature reporting the effectiveness of antimicrobial mouthwashes, particularly those containing chlorhexidine or similar agents, in reducing plaque and gingival inflammation (23). Several studies have consistently shown that such formulations provide significant reductions in bacterial counts and plaque indices over both short- and long-term use. The discrepancy observed in this study may be attributed to several factors, including the type and concentration of mouthwash used, duration of exposure, participant compliance, or methodological differences in sample collection and analysis. It is also possible that the timing of sample collection did not capture the peak antimicrobial effect of the mouthwash, thereby underestimating its efficacy.

The comparative analysis indicated that coconut oil was the most effective intervention, followed by miswak, while mouthwash showed limited effectiveness under the study conditions. These findings suggest that natural alternatives may have a role in oral hygiene practices, particularly in settings where accessibility, cost, or cultural preferences influence behavior. The results also highlight the importance of considering both short-term and sustained effects when evaluating oral hygiene interventions. Despite these findings, several limitations must be acknowledged. The study did not report the sample size or variability measures such as standard deviation, which limits the ability to generalize the results (24). The use of single representative colony counts rather than aggregated participant data reduces statistical robustness. Additionally, the standardization of TNTC values to a fixed number may introduce estimation bias. The absence of a clearly defined control group further limits the strength of comparative conclusions (25). Furthermore, only

Streptococcus mutans was evaluated, whereas dental plaque consists of a complex microbial community, and broader microbiological analysis would provide a more comprehensive understanding. The relatively short duration of the study also limits assessment of long-term effects.

Strengths of the study include the use of a randomized design, standardized sampling procedures, and the application of conventional microbiological techniques for bacterial identification and quantification. The inclusion of both natural and chemical interventions provides practical relevance and contributes to the growing interest in alternative oral hygiene strategies. Future research should focus on larger sample sizes with clearly defined control groups and include multiple bacterial species to better represent the oral microbiome. Longer study durations would help evaluate sustained effects and potential side outcomes. Additionally, standardized protocols for oil pulling and miswak usage should be established to improve reproducibility. Comparative studies incorporating advanced microbiological techniques, such as molecular analysis, may further enhance understanding of microbial changes associated with these interventions. The findings suggested that coconut oil and miswak demonstrated measurable antibacterial effects against plaque-producing bacteria, while the effectiveness of mouthwash may depend on formulation and study conditions. These results contribute to the ongoing exploration of integrating traditional and modern approaches in oral health management.

CONCLUSION

This study demonstrated that both coconut oil and miswak were effective in reducing plaque-producing bacteria, with coconut oil showing the most consistent and sustained antibacterial effect, while miswak provided a moderate yet reliable reduction over time. In contrast, the chemical mouthwash did not exhibit a lasting impact on bacterial levels under the study conditions. These findings support the potential role of natural oral hygiene practices as practical and accessible alternatives for managing oral microbial load, particularly in settings where cost, availability, or cultural preferences influence oral care choices. Overall, the study highlights the importance of integrating evidence-based traditional approaches into modern oral health strategies, while emphasizing the need for further well-designed research to validate and optimize their use in clinical practice.

AUTHOR CONTRIBUTION

Author	Contribution
Rafia Anwer*	Conceptualization, Methodology, Formal Analysis, Writing - Original Draft, Validation, Supervision
Minahil Malik	Methodology, Investigation, Data Curation, Writing - Review & Editing
Maryam Liaqat	Investigation, Data Curation, Formal Analysis, Software
Tahreem Fatima	Software, Validation, Writing - Original Draft
Rida Khalid	Formal Analysis, Writing - Review & Editing
Aqsa Siyalvi	Writing - Review & Editing, Assistance with Data Curation

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