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Evaluation of Oral Antibacterial Activity of Synthetic and Herbal Mouthwash Preparations

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Abstract

The oral cavity harbours a diverse microbial population that contributes to the development of dental plaque, caries, and periodontal diseases when oral hygiene is inadequate. Mouthwashes are widely used as adjuncts to mechanical cleaning methods to reduce oral microbial load. This study aimed to evaluate and compare the antibacterial potency of selected chemical, essential oil-based, and herbal mouthwash formulations against common oral pathogens. An in-vitro assessment was conducted using the agar well diffusion method and minimum inhibitory concentration (MIC) determination against *Streptococcus mutans* and *Lactobacillus* species. The results demonstrated that chlorhexidine-based mouthwash exhibited the highest antibacterial activity, producing the largest zones of inhibition and lowest MIC values. Essential oil-based mouthwash showed moderate antibacterial efficacy, while herbal mouthwash displayed lower but significant inhibitory effects. The findings indicate that antibacterial effectiveness varies with formulation and active ingredients. While chlorhexidine remains the most potent agent for short-term use, herbal mouthwashes offer safer alternatives for long-term oral hygiene maintenance.

Key Words: microbial, adjuncts, potent.

INTRODUCTION

The oral cavity is a complex biological environment that supports the growth of a wide range of microorganisms due to constant moisture, nutrient availability from food debris, and an optimal temperature close to body conditions. More than 700 microbial species have been identified in the oral ecosystem, many of which contribute to the development of dental plaque, gingivitis, periodontitis, dental caries, and halitosis when oral hygiene is inadequate. The accumulation of pathogenic bacteria such as *Streptococcus mutans* and *Lactobacillus* species plays a critical role in enamel demineralisation and the progression of oral infections (Grzybowska, 2013). Mechanical plaque control methods, including tooth brushing and flossing, remain essential; however, they are often insufficient to eliminate microorganisms from inaccessible areas such as interproximal spaces, gingival crevices, and the tongue surface.

Mouthwashes are widely recommended as adjuncts to mechanical oral hygiene practices due to their ability to reduce microbial load, inhibit plaque formation, and improve overall oral health. Chemical mouthwashes, particularly chlorhexidine gluconate formulations, are considered the gold standard because of their broad-spectrum antibacterial activity and prolonged substantivity in the oral cavity (Masadeh et al., 2026). Despite their effectiveness, long-term use of chemical mouthwashes has been associated with adverse effects such as tooth staining, taste alteration, mucosal irritation, and disruption of normal oral flora. These limitations have encouraged increasing interest in herbal and natural mouthwash formulations containing plant-derived compounds such as neem, clove, tea tree oil, and essential oils, which are believed to possess antimicrobial, anti-inflammatory, and antioxidant properties.

In recent years, consumer preference has shifted towards herbal mouthwashes due to their perceived safety, minimal side effects, and suitability for prolonged use. However, the antibacterial potency of herbal formulations varies depending on their composition, concentration of active ingredients, and mechanism of action. Scientific evidence comparing the antimicrobial effectiveness of chemical and herbal mouthwashes remains limited and inconsistent. Therefore, systematic evaluation of different mouthwash formulations against common oral pathogens is essential to support evidence-based recommendations for oral hygiene products. This study aims to assess and compare the antibacterial potency of selected mouthwashes to determine their effectiveness and potential role in maintaining oral health.

OBJECTIVES OF THE STUDY

1. To evaluate the antibacterial activity of selected chemical and herbal mouthwash formulations against common oral microorganisms associated with dental plaque and oral infections.

2. To compare the antimicrobial effectiveness of chemical mouthwashes, particularly chlorhexidine-based formulations, with herbal mouthwashes in terms of their inhibitory potential.

3. To assess the relative effectiveness of the selected mouthwashes against key oral bacteria, including *Streptococcus mutans* and *Lactobacillus* species, using standard in-vitro microbiological methods.

4. To identify mouthwash formulations that offer effective antibacterial action while minimizing adverse effects, making them suitable for long-term oral hygiene maintenance.

REVIEW OF LITERATURE

Extensive research has established that mouthwashes serve as effective adjuncts to mechanical plaque control methods such as tooth brushing and flossing in reducing oral bacterial load and maintaining oral health. The oral cavity hosts complex, multispecies biofilms that contribute to dental caries, gingivitis, periodontitis, and halitosis when not adequately controlled. Because mechanical methods alone may not sufficiently eliminate microorganisms from inaccessible areas such as gingival sulci and interproximal spaces, antimicrobial mouthwashes are widely recommended in preventive and therapeutic oral care. Among the various formulations available, chlorhexidine (CHX), essential oil-based, and herbal mouthwashes have been extensively studied for their antibacterial properties; however, their relative efficacy, safety, and suitability for long-term use differ considerably.

Chlorhexidine-Based Mouthwashes

Chlorhexidine gluconate is widely regarded as the “gold standard” antiseptic in mouthwash formulations due to its broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, and some viruses. According to McGrath et al. (2023), CHX exerts its antibacterial action by binding to negatively charged bacterial cell walls, increasing membrane permeability, and causing leakage of intracellular components, ultimately leading to cell death. A key advantage of CHX is its substantivity, which allows it to bind to oral tissues and tooth surfaces and be released slowly over time, providing prolonged antibacterial activity (Lim and Kam, 2008).

Clinical and in-vitro studies have consistently demonstrated the effectiveness of CHX in reducing dental plaque and gingival inflammation. Senkalvarayan et al. (2023) reported that 0.12% and 0.2% CHX mouthwashes significantly reduce plaque accumulation and gingival scores when used as adjuncts to routine oral hygiene practices. CHX has also been shown to significantly reduce counts of *Streptococcus mutans*, a primary cariogenic organism strongly associated with enamel demineralisation and dental caries progression (Russell and Day, 1993; Haffajee et al., 2008). Meta-analyses indicate substantial reductions in plaque index and gingival index following daily CHX use for periods ranging from four to six weeks.

Despite its superior antimicrobial efficacy, prolonged use of CHX is associated with adverse effects such as extrinsic tooth staining, taste alteration, mucosal irritation, and, in rare cases, hypersensitivity reactions (Gagari and Kabani, 1995). These limitations have raised concerns regarding patient compliance and have prompted interest in alternative formulations suitable for long-term use.

Essential Oil-Based Mouthwashes

Essential oil (EO) mouthwashes typically contain active compounds such as thymol, menthol, eucalyptol, and methyl salicylate, which exhibit antimicrobial and anti-inflammatory properties. These compounds act by penetrating biofilm matrices and disrupting bacterial cell membranes, leading to reduced bacterial viability and plaque formation (Trombetta et al., 2005). Unlike CHX, EO mouthwashes do not exhibit strong substantivity; however, their ability to disrupt biofilms contributes to clinically meaningful antibacterial effects.

Clinical studies reported by Quintas et al. (2015) demonstrated that EO mouthwashes are significantly more effective than placebo and mechanical plaque control alone in reducing plaque accumulation and gingival inflammation. While CHX generally provides greater plaque inhibition, EO mouthwashes often show comparable effectiveness in reducing

gingival inflammation and managing oral malodour (Hughes and McNab, 2008). Some short-term studies have reported no statistically significant difference between EO and CHX mouthwashes in plaque suppression, although CHX remains more effective during prolonged use. Importantly, EO mouthwashes are associated with fewer aesthetic side effects, making them more acceptable to many patients.

Herbal Mouthwashes

Herbal mouthwashes derived from natural products such as neem (*Azadirachta indica*), green tea, *Salvadora persica* (miswak), and propolis have gained increasing attention as safer alternatives to synthetic antiseptics. These formulations contain bioactive phytochemicals, including flavonoids, polyphenols, and terpenoids, which possess antibacterial, anti-inflammatory, and antioxidant properties (Parahitiyawa et al., 2010; Moeintaghavi et al., 2012).

Lile et al. (2025) reported that although herbal mouthwashes generally demonstrate lower antibacterial potency than 0.2% CHX in terms of maximum inhibition zones, they still significantly reduce bacterial counts and clinical indices such as plaque index (PI) and gingival index (GI) when compared with placebo. Several comparative trials have shown that certain herbal formulations perform similarly to essential oil mouthwashes and are often preferred due to fewer side effects, absence of staining, and better patient acceptance (Baradari et al., 2012).

MATERIALS AND METHODS

Sample Selection

Three different categories of mouthwash formulations were selected for this study to enable a comparative evaluation of antibacterial potency. A commercially available chemical mouthwash containing chlorhexidine gluconate (0.2%) was chosen due to its established clinical use and broad-spectrum antimicrobial activity. An essential oil-based mouthwash containing active components such as thymol, menthol, and eucalyptol was selected to represent non-chlorhexidine synthetic formulations. Additionally, a herbal mouthwash formulated with plant-based ingredients such as neem, clove, and herbal extracts was included to assess the antibacterial efficacy of natural alternatives. All mouthwash samples were obtained in sealed commercial packaging and used prior to their expiry dates.

Test Microorganisms

The antibacterial activity of the selected mouthwashes was evaluated against common oral pathogenic bacteria. Streptococcus mutans, a primary etiological agent of dental caries, and Lactobacillus species, associated with enamel demineralisation and caries progression, were selected as test organisms. Pure cultures of these microorganisms were obtained from a microbiology laboratory and maintained on appropriate culture media under standard conditions.

Methodology

The agar well diffusion method was employed to assess antibacterial activity. Mueller-Hinton agar plates were inoculated evenly with standardized bacterial suspensions. Wells of uniform diameter were prepared aseptically in the agar, and measured volumes of each mouthwash sample were added to the wells. Plates were incubated at 37 °C for 24 hours. Antibacterial efficacy was determined by measuring the zones of inhibition around each well in millimetres. The minimum inhibitory concentration (MIC) was determined using serial dilution techniques to identify the lowest concentration of mouthwash that inhibited visible bacterial growth.

Statistical Analysis

All experiments were performed in triplicate. The data obtained were analysed using descriptive statistics, and mean values of inhibition zones were calculated. Comparative analysis was conducted to evaluate differences in antibacterial activity among the tested mouthwashes.

RESULTS

The antibacterial activity of the selected mouthwash formulations was evaluated against *Streptococcus mutans* and *Lactobacillus* species using the agar well diffusion method and minimum inhibitory concentration (MIC) determination. The results showed clear variation in antibacterial potency among chemical, essential oil-based, and herbal mouthwashes.

Table 1: Zone of Inhibition (mm) Against Oral Bacteria (Mean ± SD)

Mouthwash Type	*Streptococcus mutans* (mm)	*Lactobacillus* spp. (mm)
Chlorhexidine (0.2%)	24.6 ± 1.2	22.9 ± 1.0
Essential Oil-based	18.4 ± 1.1	16.7 ± 0.9
Herbal Mouthwash	13.2 ± 0.8	11.9 ± 0.7

The chlorhexidine-based mouthwash produced the largest zones of inhibition against both microorganisms, indicating the highest antibacterial potency. The essential oil-based mouthwash showed moderate inhibition, while the herbal mouthwash demonstrated lower but clearly measurable antibacterial activity.

Table 2: Minimum Inhibitory Concentration (MIC)

Mouthwash Type	MIC for *S. mutans* (µg/mL)	MIC for *Lactobacillus* spp. (µg/mL)
Chlorhexidine	0.6	0.8
Essential Oil-based	1.8	2.2
Herbal Mouthwash	3.5	4.0

Chlorhexidine required the lowest concentration to inhibit bacterial growth, confirming its superior efficacy. Herbal mouthwash required the highest concentration, indicating comparatively weaker antibacterial activity.

DISCUSSION

The results of this study clearly demonstrate that antibacterial efficacy varies significantly depending on the formulation and active ingredients of mouthwashes. The superior performance of the chlorhexidine-based mouthwash can be attributed to its broad-spectrum antimicrobial mechanism and high substantivity. Chlorhexidine binds strongly to bacterial cell walls and oral tissues, allowing sustained release and prolonged antibacterial action. This explains both the larger zones of inhibition and lower MIC values observed against *Streptococcus mutans* and *Lactobacillus* species.

The essential oil-based mouthwash exhibited moderate antibacterial activity. The presence of bioactive compounds such as thymol, menthol, and eucalyptol likely contributed to membrane disruption and biofilm penetration. Although its inhibition zones were smaller than chlorhexidine, essential oil formulations demonstrated consistent antibacterial action against both organisms. Their moderate MIC values suggest adequate antimicrobial effectiveness while avoiding the adverse effects associated with prolonged chlorhexidine use.

The herbal mouthwash, while showing the lowest antibacterial potency, still produced statistically meaningful inhibition zones. This activity can be attributed to phytochemicals such as flavonoids, tannins, and terpenoids present in herbal extracts like neem and clove. These compounds exert antimicrobial and anti-inflammatory effects, though their concentration and substantivity are generally lower than synthetic agents. Importantly, herbal mouthwashes are associated with fewer side effects such as tooth staining, taste alteration, and mucosal irritation.

Overall, the findings support existing literature indicating that chlorhexidine is most suitable for short-term therapeutic use, particularly in managing acute plaque-related conditions. However, essential oil and herbal mouthwashes offer safer alternatives for long-term oral hygiene maintenance, balancing antibacterial efficacy with improved patient compliance and reduced adverse effects.

CONCLUSION

The present study systematically evaluated the antibacterial potency of chemical, essential oil-based, and herbal mouthwash formulations against common oral microorganisms, namely *Streptococcus mutans* and *Lactobacillus* species. The findings clearly demonstrate that chlorhexidine-based mouthwash exhibits the highest antibacterial efficacy, as evidenced by the largest zones of inhibition and the lowest minimum inhibitory concentration values. This superior performance can be attributed to chlorhexidine's broad-spectrum antimicrobial activity and its substantivity, which allows prolonged retention and sustained antibacterial action in the oral cavity. These properties make chlorhexidine particularly effective for short-term therapeutic use in managing dental plaque, gingivitis, and other plaque-related oral infections.

Despite its high efficacy, the study also highlights important considerations regarding the routine use of chlorhexidine mouthwash. Long-term use is associated with undesirable effects such as tooth staining, taste alteration, and mucosal irritation, which may reduce patient compliance. In contrast, herbal mouthwashes demonstrated lower but significant antibacterial activity, confirming the presence of biologically active phytochemicals capable of inhibiting oral pathogens. Although less potent than chlorhexidine, herbal formulations offer advantages such as improved safety, reduced side effects, and greater suitability for long-term daily oral hygiene maintenance. Essential oil-based mouthwashes were found to provide a balanced profile, offering moderate antibacterial efficacy with fewer adverse effects than chlorhexidine. Overall, the study supports a selective approach to mouthwash use, in which chlorhexidine is recommended for short-term therapeutic applications, while herbal and essential oil mouthwashes may be preferred for routine oral care.

Limitations of the study

The study has certain limitations that should be acknowledged. First, the antibacterial evaluation was conducted under in-vitro laboratory conditions, which may not accurately replicate the complex biological environment of the oral cavity, including saliva flow, biofilm formation, and host immune responses. Second, only a limited number of commercially available mouthwash formulations were tested, which may restrict the generalisability of the findings. Additionally, the study focused solely on antibacterial activity and did not assess other clinically relevant parameters such as anti-inflammatory effects, taste acceptability, or patient compliance.

Future scope

Future research should focus on clinical trials involving human participants to validate the in-vitro findings under real oral conditions. Long-term studies are required to evaluate the sustained effectiveness, safety, and patient compliance associated with herbal and essential oil-based mouthwashes. Further investigation into the standardisation and optimisation of herbal formulations may enhance their antibacterial potency while preserving safety. Development of novel mouthwash formulations that combine synthetic and herbal agents could offer improved efficacy with minimal adverse effects, contributing to more effective and sustainable oral healthcare solutions.

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