Alqalam Journal of Medical and Applied Sciences. 2026;9(9):2640-2645
https://doi.org/10.54361/ajmas.269901

Original article

Antimicrobial Activity of Mouthwashes Against Gingival Bacteria

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Abstract
Gingivitis is a common inflammatory oral disease caused primarily by the accumulation of dental plaque and associated bacterial pathogens. Mouthwashes are widely used as adjunctive agents for controlling oral microorganisms and preventing periodontal disease progression. This study aimed to evaluate the antibacterial activity of different mouthwash formulations, including Cariax Chlorhexidine DG Fluoride (0.12%), Perio Chlorhexidine DG (0.20%) with 0.1% cetylpyridinium chloride (CPC), 3% hydrogen peroxide (H_2O_2), and nystatin, against gingival-associated bacterial pathogens. The antimicrobial effects of the tested formulations were evaluated *against Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, and Escherichia coli* using the disc diffusion method. Sterile filter paper discs impregnated with the tested agents were used to determine inhibition zones. The results demonstrated that 3% hydrogen peroxide exhibited the strongest antibacterial activity against all tested bacterial isolates, producing the largest mean inhibition zones against *P. aeruginosa* (23 mm), *S. aureus* (41 mm), *S. epidermidis* (25 mm), *K. pneumoniae* (29 mm), and *E. coli* (25 mm), with statistically significant differences compared with other agents. Chlorhexidine formulations showed moderate antibacterial activity, with the 0.20% concentration generally producing greater inhibition than the 0.12% formulation. As expected, nystatin, included as an antifungal negative control, showed no antibacterial activity against the tested bacterial species. These findings indicate that 3% hydrogen peroxide has strong in *vitro* antibacterial potential against common gingival pathogens, while chlorhexidine remains an effective antimicrobial agent with prolonged activity. Further clinical studies are required to confirm the effectiveness of these formulations in patients with gingivitis and periodontal disease.
Keywords. Gingivitis, Mouthwash, Hydrogen Peroxide and Chlorhexidine.

Introduction

Gingivitis is a reversible inflammatory condition of the gingival tissues characterized by a site-specific host inflammatory response to the accumulation of dental plaque (biofilm) at the gingival margin [1, 2]. Unlike periodontitis, gingivitis does not involve destruction of the periodontal ligament or resorption of the supporting alveolar bone [2]. Therefore, it represents an early stage of periodontal disease and an important opportunity for prevention and intervention before irreversible periodontal tissue damage occurs. Gingivitis is highly prevalent worldwide, with epidemiological studies indicating that up to 90% of adults may experience some degree of gingival inflammation [3]. Although it can occur at any age, its prevalence and severity may increase during periods of hormonal fluctuation, such as puberty and pregnancy, when gingival tissues become more susceptible to plaque-induced inflammation [4]. Effective control of dental plaque is essential for preventing and managing gingivitis. In addition to mechanical plaque removal, antimicrobial mouthwashes are widely used as adjunctive oral hygiene agents. Commercial mouthwashes differ in their active ingredients, antimicrobial spectrum, efficacy, and adverse-effect profiles.

Chlorhexidine (CHX) is among the most extensively studied agents for chemical plaque control and is considered highly effective because of its broad-spectrum antimicrobial activity and high substantivity, which allows prolonged antimicrobial activity within the oral cavity following rinsing [5, 6]. Essential oil-containing mouthrinses, including formulations containing eucalyptol, menthol, and thymol, have also demonstrated efficacy during long-term use, with significant reductions in dental plaque and gingival inflammation when used in conjunction with mechanical plaque control [7]. Cetylpyridinium chloride (CPC), a quaternary ammonium compound commonly incorporated into alcohol-free mouthwash formulations, has similarly demonstrated plaque- and gingivitis-reducing effects when used as an adjunct to toothbrushing [8, 9]. Hydrogen peroxide (H_2O_2) is another antimicrobial agent used in oral care products. As an oxidizing agent, H_2O_2 exerts antimicrobial effects through oxidative damage to cellular components and may inhibit the colonization and multiplication of anaerobic bacteria within dental plaque [10, 11].

In addition to differences in antimicrobial activity, mouthwash formulations may vary in their effects on the oral microbiome and in their acceptability to patients [12, 13]. These differences highlight the importance of evaluating the antibacterial activity of individual formulations against clinically relevant microorganisms. The microbial composition associated with gingival inflammation may include a variety of Gram-positive and Gram-negative bacterial species. The presence and proliferation of potentially pathogenic microorganisms may contribute to the persistence of gingival inflammation, particularly when effective plaque control is inadequate [14, 15]. In developing countries, limited access to professional dental care, inadequate oral health education, poverty, and insufficient oral healthcare resources may further contribute to the burden of oral and periodontal diseases [16]. Consequently, accessible and effective antimicrobial approaches may have an important role as adjuncts to routine oral hygiene practices. Despite the widespread availability of antimicrobial mouthwashes, differences in their antibacterial activity against specific oral and opportunistic bacterial species warrant further investigation. Therefore, the present study aimed to evaluate and compare the in *vitro* antibacterial activity of

Received: *25-06-2026 -* ***Accepted:*** *23-08-2026 -* ***Published:*** *01-09-2026*

selected mouthwash formulations, including Cariax Chlorhexidine DG Fluoride (0.12%), Perio Chlorhexidine DG (0.20%) containing 0.1% cetylpyridinium chloride (CPC), 3% hydrogen peroxide (H_2O_2), and nystatin. Their antibacterial activity was evaluated against *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae,* and *Escherichia coli* using laboratory-based antibacterial susceptibility testing.

Methods

This study employed a laboratory-based in vitro experimental design conducted under controlled conditions. Sample collection and laboratory analyses were performed between January and February 2026 in western Libya, with standardized procedures applied throughout sample handling and experimental processing. The antimicrobial activities of four oral formulations were evaluated: Cariax Chlorhexidine DG Fluoride (0.12%), Perio Chlorhexidine DG (0.20%) containing 0.1% cetylpyridinium chloride (CPC), 3% hydrogen peroxide (H_2O_2) spray, and Nystatin oral suspension (100,000 IU/mL; 30 mL). Nystatin was included as an antifungal negative control because its primary activity is directed against fungal organisms rather than bacteria. The tested formulations were evaluated against five bacterial species: *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae,* and *Escherichia coli*. For the antimicrobial susceptibility assay, sterile filter paper discs measuring 6 mm in diameter were prepared from Whatman® No. 1 filter paper (Sigma-Aldrich). The discs were placed in heat-sealing bags and sterilized by autoclaving at 121°C for 15 minutes.
Following sterilization, the discs were dried in a hot-air oven at 50°C to remove residual moisture. The sterile discs were subsequently impregnated with the respective test formulations and used for antimicrobial susceptibility testing under standardized laboratory conditions. The bacterial colonies were suspended in 0.9% normal saline, and the bacterial density was adjusted to the 0.5 McFarland standard using a spectrophotometer. The standardized bacterial suspension was then evenly inoculated onto the entire surface of Mueller-Hinton agar (CM0337) using a sterile swab. Antibiotic discs were placed on the inoculated agar surface, followed by the prepared mouthwash-impregnated discs. The mouthwash discs were prepared by immersing sterile filter paper discs in their respective mouthwash formulations and then placing them evenly on the agar plates. The experiment was performed in triplicate for each bacterial species to minimize experimental error. The plates were incubated at 37°C for 24 hours. Finally, the diameters of the bacterial inhibition zones were measured in millimetres, and the results were recorded for subsequent statistical analysis. GraphPad Prism 9.4.1 software (2022, U.S.A. was used to analyse the data.

Results

Hydrogen peroxide (H_2O_2, 3%) demonstrated the highest antibacterial activity against *Pseudomonas aeruginosa*, producing the largest mean inhibition zone (23 mm), which was significantly greater than that observed for the other tested agents (****$P < 0.0001$). Chlorhexidine showed moderate antibacterial effects, with 0.20% and 0.12% formulations yielding mean inhibition zones of approximately 18 mm and 12 mm, respectively. In contrast, as expected for the antifungal negative control, nystatin showed no detectable antibacterial activity against *P. aeruginosa*.

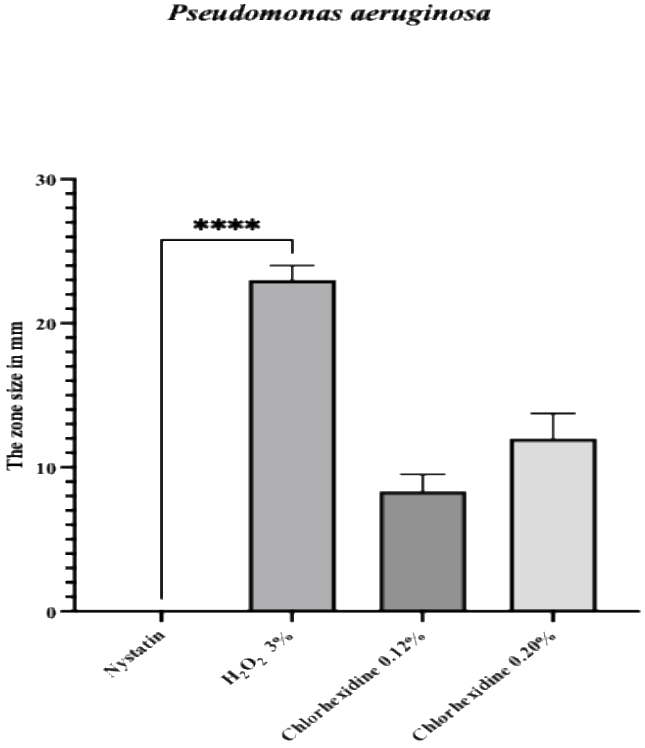

Figure 1. Antibacterial activity against *Pseudomonas aeruginosa*

H_2O_2 (3%) produced the largest mean inhibition zone against *Staphylococcus aureus* (41 mm), which was significantly greater than those produced by the other tested agents (***$P < 0.0002$). Chlorhexidine also inhibited *S. aureus* growth, with

Received: *25-06-2026 -* ***Accepted:*** *23-08-2026 -* ***Published:*** *01-09-2026*

mean inhibition zones of 19 mm and 15 mm for the 0.20% and 0.12% formulations, respectively. No inhibitory activity was observed for nystatin against *S. aureus*.

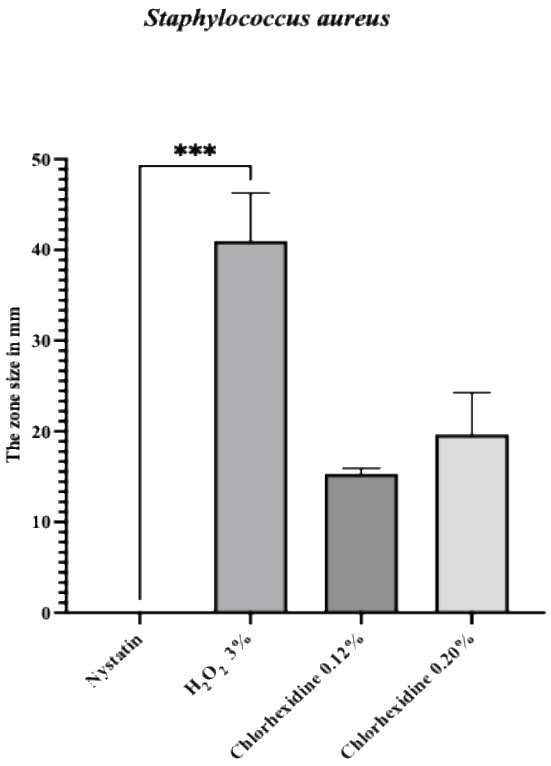

Figure 2. Antibacterial activity against *Staphylococcus aureus*

H_2O_2 (3%) exhibited the highest antibacterial activity against *Staphylococcus epidermidis*, producing the largest mean inhibition zone (25 mm). This inhibitory effect was significantly greater than those observed for the other tested mouthwash formulations (*$P < 0.0214$). Chlorhexidine formulations (0.12% and 0.20%) demonstrated moderate antibacterial activity, with mean inhibition zones of approximately 18 mm and 19 mm, respectively. In contrast, Nystatin, included as the antifungal negative control, showed no measurable antibacterial activity against *S. epidermidis*.

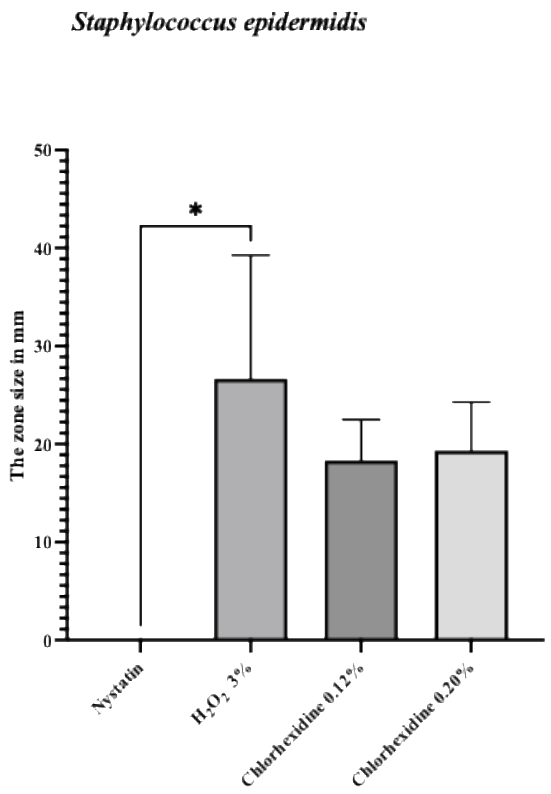

Fig 3. Antibacterial activity against *Staphylococcus epidermidis*

Received: *25-06-2026 -* ***Accepted:*** *23-08-2026 -* ***Published:*** *01-09-2026*

H_2O_2 (3%) demonstrated the highest antibacterial activity against *K. pneumoniae*, producing the largest mean inhibition zone (29 mm), which was significantly greater than those observed for the other tested agents (***$P < 0.0006$). Chlorhexidine formulations (0.12% and 0.20%) exhibited moderate antibacterial activity, with mean inhibition zones of approximately 10 mm and 13 mm, respectively. In contrast, Nystatin showed no detectable antibacterial activity against *K. pneumoniae*, consistent with its role as an antifungal negative control.

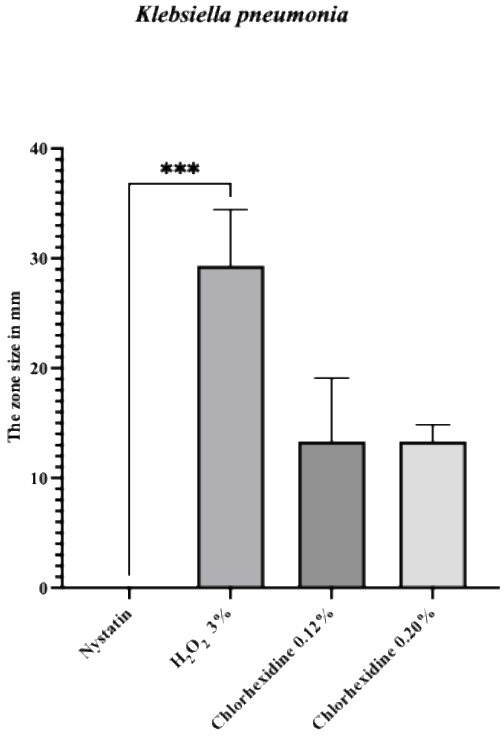

Fig 4. Antibacterial activity against *Klebsiella pneumoniae*

H_2O_2 (3%) showed the greatest antibacterial activity against *Escherichia coli*, producing the largest mean inhibition zone (25 mm), which was significantly higher than the other tested agents (****$P < 0.0001$). Chlorhexidine 0.12% and 0.20% exhibited moderate antibacterial activity with mean inhibition zones of approximately 13 mm and 12 mm, respectively, while nystatin, used as an antifungal negative control, showed no inhibitory effect against *E. coli*.

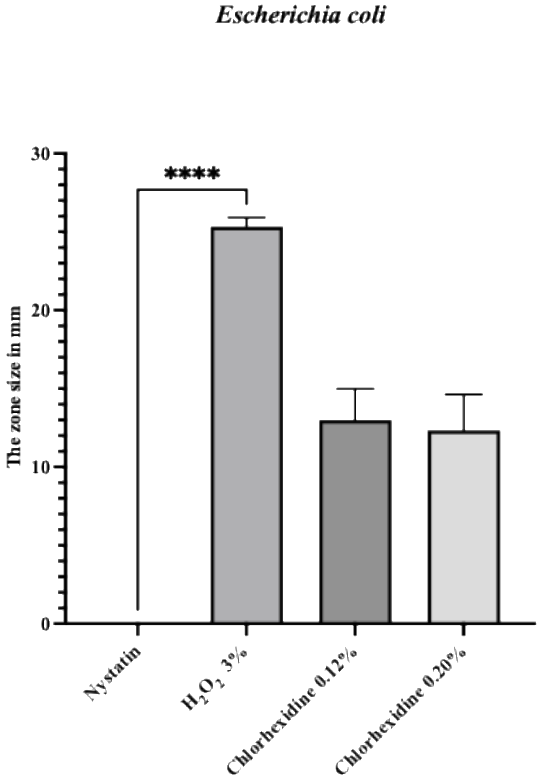

Fig.5. Antibacterial activity against *Escherichia coli*

Received: *25-06-2026 -* ***Accepted:*** *23-08-2026 -* ***Published:*** *01-09-2026*

Discussion

The present study evaluated and compared the in vitro antibacterial activity of selected mouthwash formulations against bacterial isolates associated with gingival infections. Among the tested agents, 3% hydrogen peroxide (H_2O_2) demonstrated the strongest antibacterial activity, producing the largest inhibition zones against *Pseudomonas aeruginosa* (23 mm), *Staphylococcus aureus* (41 mm), *Staphylococcus epidermidis* (25 mm), *Klebsiella pneumoniae* (29 mm), and *Escherichia coli* (25 mm). The observed differences were statistically significant, indicating that H_2O_2 exhibited greater antibacterial activity than the other tested formulations under the experimental conditions of this study.

Antibacterial Activity of Hydrogen Peroxide

The strong antibacterial activity of hydrogen peroxide is primarily attributed to oxidative stress and the generation of reactive oxygen species (ROS), which can damage essential cellular components, including proteins, lipids, and nucleic acids, ultimately leading to loss of cellular integrity and bacterial death. This oxidative mechanism contributes to the broad-spectrum antimicrobial activity of hydrogen peroxide against both Gram-positive and Gram-negative bacteria [17, 18]. These findings are consistent with previous research demonstrating that 3% hydrogen peroxide significantly reduces bacterial counts on the tongue, supporting its potential antimicrobial effect in reducing the oral bacterial burden [19]. However, further clinical studies are needed to confirm its efficacy under in *vivo* conditions. The relatively large inhibition zones observed with H_2O_2 against all bacterial isolates suggest that its oxidative activity was effective against a broad range of microorganisms under the present in *vitro* conditions.

Antibacterial Activity of Chlorhexidine

Both chlorhexidine formulations exhibited moderate antibacterial activity against all tested bacteria. The 0.20% chlorhexidine formulation generally produced larger inhibition zones than the 0.12% formulation against *P. aeruginosa*, *S. aureus*, and *K. pneumoniae*, suggesting that increasing chlorhexidine concentration enhances its antibacterial effect. However, against *S. epidermidis* and *E. coli*, the difference between the two formulations was minimal. Chlorhexidine exerts its antimicrobial activity by disrupting the bacterial cell membrane, causing leakage of intracellular contents and inhibition of essential cellular processes. Although its inhibition zones were smaller than those observed with hydrogen peroxide, chlorhexidine remains one of the most effective and widely studied agents for chemical plaque control because of its high substantivity and prolonged antimicrobial activity following application. However, its clinical use may be limited by adverse effects, particularly extrinsic tooth staining and taste alteration [9, 20, 21].

Lack of Antibacterial Activity of Nystatin

Nystatin showed no detectable antibacterial activity against any of the bacterial isolates tested. In the present study, nystatin was intentionally included as an antifungal negative control rather than as an antibacterial mouthwash. This lack of antibacterial activity was expected because nystatin is a polyene antifungal agent that primarily acts against *Candida* species. Its mechanism of action involves binding to ergosterol in fungal cell membranes, resulting in pore formation and leakage of cellular contents. Since bacterial cell membranes lack ergosterol, nystatin has no significant antibacterial effect. Therefore, its absence of activity against *P. aeruginosa, S. aureus, S. epidermidis, K. pneumoniae,* and *E. coli* agrees with its established pharmacological properties [22, 23]. Overall, the findings indicate that hydrogen peroxide may provide rapid and effective reduction of oral bacterial pathogens under in vitro conditions, whereas chlorhexidine provides moderate antibacterial activity with the additional advantage of prolonged antimicrobial substantivity. The absence of antibacterial activity with nystatin confirms its specific role as an antifungal rather than an antibacterial agent. Further clinical studies are recommended to determine whether the superior in *vitro* antibacterial activity of hydrogen peroxide translates into improved clinical outcomes in patients with gingivitis and periodontal disease.

Limitations

This *in vitro* study requires confirmation through larger *in vivo* clinical studies.

Conclusion

This study demonstrated that 3% hydrogen peroxide exhibited the highest antibacterial activity against all tested gingival bacterial pathogens, producing significantly larger inhibition zones than the other tested agents. Chlorhexidine formulations showed moderate antibacterial effects, whereas nystatin, included as an antifungal negative control, showed no antibacterial activity, as expected. These findings suggest that hydrogen peroxide has strong in *vitro* antibacterial potential, whereas chlorhexidine remains valuable for its prolonged antimicrobial action. Further clinical studies are needed to confirm these effects in patients.

Acknowledgments

The authors thank the staff of the participating clinics and the Faculty of Medical Technology-Surman, Sabratha University, for their support and assistance during this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Received*: 25-06-2026 -* ***Accepted****: 23-08-2026 -* ***Published****: 01-09-2026*

References

1. Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, Geisinger ML, Genco RJ, Glogauer M, Goldstein M, Griffin TJ, Holmstrup P, Johnson GK, Kapila Y, Lang NP, Meyle J, Murakami S, Plemons J, Romito GA, Shapira L, Tatakis DN, Teughels W, Trombelli L, Walter C, Wimmer G, Xenoudi P, Yoshie H. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol. 2018 Jun;45 Suppl 20:S68-S77. doi: 10.1111/jcpe.12940. PMID: 29926499.
2. Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, Geisinger ML, Genco RJ, Glogauer M, Goldstein M, Griffin TJ, Holmstrup P, Johnson GK, Kapila Y, Lang NP, Meyle J, Murakami S, Plemons J, Romito GA, Shapira L, Tatakis DN, Teughels W, Trombelli L, Walter C, Wimmer G, Xenoudi P, Yoshie H. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Clin Periodontol. 2018;45(Suppl 20):S68-S77.
3. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005;366(9499):1809-1820.
4. Marsh PD, Zaura E. Dental biofilm: ecological interactions in health and disease. J Clin Periodontol. 2017;44(Suppl 18):S12-S22.
5. Elworthy A, Greenman J, Doherty FM, Newcombe RG, Addy M. The substantivity of a number of oral hygiene products determined by the duration of effects on salivary bacteria. J Periodontol. 1996;67(6):572-576.
6. Tomás I, Cousido MC, García-Caballero L, Rubido S, Limeres J, Diz P. Substantivity of a single chlorhexidine mouthwash on salivary flora: influence of intrinsic and extrinsic factors. J Dent. 2010;38(7):541-546.
7. Araujo MWB, Charles CA, Weinstein RB, McGuire JA, Parikh-Das AM, Du Q, Zhang J, Berlin JA, Gunsolley JC. Meta-analysis of the effect of an essential oil-containing mouthrinse on gingivitis and plaque. J Am Dent Assoc. 2015;146(8):610-622.
8. Ayad F, Prado R, Mateo LR, Stewart B, Szewczyk G, Arvanitidou E, Panagakos FS. A comparative investigation to evaluate the clinical efficacy of an alcohol-free CPC-containing mouthwash as compared to a control mouthwash in controlling dental plaque and gingivitis: a six-month clinical study on adults in San Jose, Costa Rica. J Clin Dent. 2011;22(6):204-212.
9. Oo MMT, Oo PH, Saddki N. Efficacy of 0.05% cetylpyridinium chloride mouthwash as an adjunct to toothbrushing compared with 0.12% chlorhexidine gluconate mouthwash in reducing dental plaque and gingival inflammation: A randomized control trial. Int J Dent Hyg. 2023;21(1):195-202.
10. Wennström J, Lindhe J. Effect of hydrogen peroxide on developing plaque and gingivitis in man. J Clin Periodontol. 1979;6(2):115-130.
11. Finnegan M, Linley E, Denyer SP, McDonnell G, Simons C, Maillard JY. Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms. J Antimicrob Chemother. 2010;65(10):2108-2115.
12. Brookes Z, Teoh L, Cieplik F, Kumar P. Mouthwash Effects on the Oral Microbiome: Are They Good, Bad, or Balanced? Int Dent J. 2023;73(Suppl 2):S74-S81.
13. Wattanawongwan W, Krasaesin A, Khieota T, Thongchotchat V, Porntaveetus T, Wiriyakijja P. Effects of chlorhexidine and a polyherbal mouthwash on the oral microbiome and user satisfaction: a randomized controlled trial. Clin Oral Investig. 2025;29(12):555.
14. van der Ploeg GR, Brandt BW, Keijser BJF, van der Veen MH, Volgenant CMC, Zaura E, Smilde AK, Westerhuis JA, Heintz-Buschart A. Multi-way modelling of oral microbial dynamics and host-microbiome interactions during induced gingivitis. NPJ Biofilms Microbiomes. 2024;10(1):89.
15. Murakami S, Mealey BL, Mariotti A, Chapple ILC. Dental plaque-induced gingival conditions. J Periodontol. 2018;89(Suppl 1):S17-S27.
16. Pack AR. Dental services and needs in developing countries. Int Dent J. 1998 Jun;48(3 Suppl 1):239-47. doi: 10.1111/j.1875-595x.1998.tb00712. x. PMID: 9779104.
17. Finnegan M, Linley E, Denyer SP, McDonnell G, Simons C, Maillard JY. Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms. J Antimicrob Chemother. 2010 Oct;65(10):2108-15. doi: 10.1093/jac/dkq308. Epub 2010 Aug 16. PMID: 20713407.
18. Lam PL, Wong RS, Lam KH, Hung LK, Wong MM, Yung LH, Ho YW, Wong WY, Hau DK, Gambari R, Chui CH. The role of reactive oxygen species in the biological activity of antimicrobial agents: An updated mini review. Chem Biol Interact. 2020 Apr 1; 320:109023. doi: 10.1016/j.cbi.2020.109023. Epub 2020 Feb 22. PMID: 32097615.
19. Soutome S, Otsuru M, Hayashida S, Naruse T, Morishita K, Kurihara K, Kawashita Y, Funahara M, Umeda M, Taniguchi H, Saito T. Efficacy of 3% hydrogen peroxide solution in cleaning tongue coating before and after surgery: a randomized phase II study. BMC Oral Health. 2022 Jul 15;22(1):287. doi: 10.1186/s12903-022-02325-9. PMID: 35841016; PMCID: PMC9288054.
20. Andrucioli MCD, Ferreira Amato PA, Kuchler ÉC, Matsumoto MAN, Bergamo AZN, Silva RABD, Silva LABD, Nelson-Filho P. Effect of chlorhexidine mouthwashes on periodontal parameters and extrinsic tooth staining in orthodontic patients. Am J Orthod Dentofacial Orthop. 2023 Dec;164(6):855-861. doi: 10.1016/j.ajodo.2023.05.034. Epub 2023 Aug 30. PMID: 37642605.
21. Brookes ZLS, McCullough M, Kumar P, McGrath C. Mouthwashes: Implications for Practice. Int Dent J. 2023 Nov;73 Suppl 2(Suppl 2): S98-S101. doi: 10.1016/j.identj.2023.08.013. Epub 2023 Oct 19. PMID: 37867062; PMCID: PMC10690539.
22. Szomek M, Reinholdt P, Petersen D, Caci A, Kongsted J, Wüstner D. Direct observation of nystatin binding to the plasma membrane of living cells. Biochim Biophys Acta Biomembr. 2021 Feb 1;1863(2):183528. doi: 10.1016/j.bbamem.2020.183528. Epub 2020 Dec 3. PMID: 33279513.
23. Récamier KS, Hernández-Gómez A, González-Damián J, Ortega-Blake I. Effect of membrane structure on the action of polyenes: I. Nystatin action in cholesterol- and ergosterol-containing membranes. J Membr Biol. 2010 Sep;237(1):31-40. doi: 10.1007/s00232-010-9304-z. Epub 2010 Sep 26. PMID: 20872217.

Received*: 25-06-2026 -* ***Accepted****: 23-08-2026 -* ***Published****: 01-09-2026*