

Original Research Article

Comparative antibacterial efficacy of a commercially available herbal mouthwash and chlorhexidine mouthwash against *Aggregatibacter actinomycetemcomitans*: an *in vitro* study

Leina R. Pradhan, Kalpna Chaudhry*, Nitin Khanduri, Yoshita Gupta, Chandni Dhyani

Department of Pediatrics and Preventive Dentistry, Seema Dental College & Hospital, Rishikesh, Uttarakhand, India

Received: 02 January 2026

Accepted: 06 February 2026

*Correspondence:

Dr. Kalpna Chaudhry,

E-mail: kkalpna78@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: Dental caries and periodontal diseases are widespread oral health problems caused primarily by plaque-forming microorganisms. While chlorhexidine (CHX) is widely used and highly effective as a chemical plaque-control agent, its prolonged use is limited by side effects such as staining and altered taste. This has led to growing interest in herbal mouthwashes, which offer antimicrobial benefits with fewer adverse effects, making them a promising natural alternative for long-term oral hygiene. To compare the antibacterial efficacy of a herbal mouthwash (Turmwash™) with chlorhexidine (Hexidine™) against *Actinomyces actinomycetemcomitans* using agar well diffusion and minimum inhibitory concentration (MIC) assays.

Methods: *A. actinomycetemcomitans* (ATCC 29522) was revived and cultured. Antibacterial activity of Turmwash™ and Hexidine™ was assessed by measuring zones of inhibition (ZOI) on Mueller–Hinton agar. MIC was determined using two-fold serial dilutions (1000–1.95 µg/mL) in Mueller–Hinton broth, followed by resazurin-based growth confirmation. Data were analyzed using the Wilcoxon signed-rank test, with $p < 0.05$ considered significant.

Results: Mean ZOI values for Turmwash™ ranged from 20–22 mm, while Hexidine™ showed ZOI values of 24–25 mm. Statistical analysis revealed no significant difference between the two ($p = 0.125$). MIC assays demonstrated that Turmwash™ inhibited bacterial growth between 15.62 µg/ml and 31.25 µg/mL, with higher concentrations showing complete inhibition.

Conclusions: Although Hexidine™ exhibited slightly higher antibacterial activity, the difference compared with the herbal mouthwash was not statistically significant. Turmwash™ demonstrated substantial antibacterial efficacy against *A. actinomycetemcomitans*, suggesting it may serve as a potential natural alternative to chlorhexidine with reduced risk of side effects.

Keywords: Herbal mouthwash, Chlorhexidine, *A. actinomycetemcomitan*, Antibacterial activity, Pediatric dentistry

INTRODUCTION

Dental caries and periodontal diseases continue to be among the most pervasive global oral health problems. A primary contributing factor is dental plaque; a highly organized biofilm composed of diverse microbial communities. While mechanical plaque control through tooth brushing is fundamental to oral hygiene, its effectiveness can be limited by improper technique, lack of compliance, and incomplete plaque removal.¹

Consequently, chemical plaque control methods particularly mouthwashes are widely employed as adjuncts to mechanical cleaning. *Actinomyces* species are Gram-positive, filamentous bacteria that play an important role in the early stages of dental plaque formation.² They are among the first microorganisms to colonize the tooth surface, adhering strongly to the acquired pellicle through specialized adhesins. Once established, *Actinomyces* create a foundation that facilitates the attachment of other bacterial species,

contributing to the structural development and maturation of plaque biofilm. Their ability to form cohesive colonies and interact with other oral bacteria promotes plaque stability and increases the risk of dental caries and periodontal inflammation.³ Some species, such as *Actinomyces actinomycetemcomitans*, are associated with periodontal disease due to their capacity to invade tissues and induce inflammatory responses, further highlighting their significance in oral microbial ecology.⁴ CHX has long been regarded as the gold standard among chemical plaque-control agents due to its broad-spectrum antibacterial properties and substantivity.⁵ However, extended use of CHX is commonly associated with adverse effects, including tooth staining, altered taste perception, oral mucosal irritation, and, in some cases, increased calculus formation.⁶ These limitations have prompted the search for safer, well-tolerated alternatives.

In recent years, herbal mouthwashes have gained significant popularity due to their natural origin, lower incidence of side effects, and multiple therapeutic properties. Herbal formulations often incorporate plant-derived ingredients such as turmeric, neem, tulsi, clove, and mint—each known for antimicrobial, anti-inflammatory, and antioxidant effects.⁷ The absence of alcohol and added sugars further enhances their safety profile and acceptability. As a result, herbal mouthwashes have emerged as promising candidates for sustainable and holistic oral care.

This study aimed to compare the antibacterial efficacy of a herbal mouthwash (Turmwash™) with that of an established chlorhexidine mouthwash (Hexidine™) against *Actinomyces actinomycetemcomitans*, a key pathogen implicated in periodontal disease.

METHODS

Study design

This in vitro experimental study was conducted to evaluate and compare the antimicrobial activity of a herbal mouthwash (Turmwash™) and chlorhexidine mouthwash (Hexidine™) against *A. actinomycetemcomitans*.

Ethical approval

Before commencement, the study was submitted for approval and clearance was obtained from Scientific Review Board, Seema Dental College & Hospital, Rishikesh EC/NEW/ INST/2025/UA/0615. The study was carried out in the DNA-Labs CRIS (Centre for Research & Innovative Studies), Dehradun.

Materials

Two commercially available mouthwashes were procured from an authorized pharmacy. Turmwash–Herbal

formulation (experimental mouthwash). Hexidine–Chlorhexidine-based formulation (control mouthwash).

Bacterial strain and revival

A. actinomycetemcomitans (ATCC 29522) was acquired from Embee Diagnostics Pvt. Ltd., New Delhi, stored at 2–4°C, and transported to DNA Labs–CRIS, Dehradun, for revival. Revival was completed within 48–72 hours following recommended biosafety guidelines. Cultures were grown overnight in Luria-Bertani (LB) broth. Mueller–Hinton agar (MHA) plates were prepared for antimicrobial testing.

Agar well diffusion assay

The antibacterial activity of each mouthwash was evaluated using the agar well diffusion method. A standardized bacterial suspension was evenly spread across Mueller–Hinton agar plates using sterile swabs to ensure uniform growth. Wells measuring 6 mm in diameter were then created in the agar using a sterile borer, and each well was filled with 50 µl of either Turmwash™, Hexidine™, the positive control, or the negative control (Figure 1). The inoculated plates were incubated at 37°C for 24–48 hours in a 5% CO₂ atmosphere to allow for optimal bacterial growth and antimicrobial activity. After incubation, the zones of inhibition surrounding each well were measured in millimetres using a Vernier calliper, with the diameter of the well included in the measurement.

Minimum inhibitory concentration assay

MIC was determined using two-fold serial dilutions of each mouthwash (1000–1.95 µg/ml) in Mueller–Hinton broth. Each well of a 96-well microtiter plate received 100 µl of diluted mouthwash and 100 µl of a 10⁵ CFU/ml bacterial suspension.

Plates were incubated at 37°C with 5% CO₂ for 24 hours. Visible turbidity was used as the primary indicator of growth. A resazurin dye assay confirmed results Blue→No bacterial growth, Pink→Bacterial growth

Statistical analysis

All data were analyzed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and are presented as mean ± standard deviation (SD). The Wilcoxon signed-rank test was used to compare ZOI values between the two mouthwashes. A *p* value < 0.05 was considered statistically significant.

RESULTS

Zone of inhibition

Wilcoxon signed-rank test: *p* = 0.125. Since *p* > 0.05, (Table 1) the difference between the two groups is not

statistically significant. Although Hexidine™ demonstrated slightly larger inhibition zones, Turmwash™ showed comparable antibacterial activity (Figure 2).

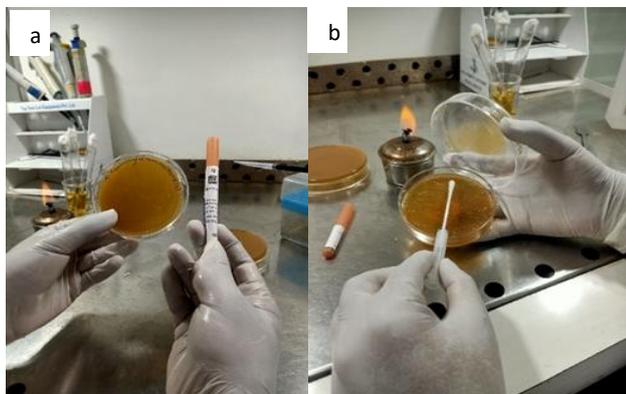


Figure 1 (a, b): Steps using well diffusion method in which plate is prepared, swab inoculation and bacterial spreading is done.



Figure 2: ZOI comparison.

Minimum inhibitory concentration

The MIC of Turmwash™ was found between 15.62 µg/mL and 31.25 µg/ml, representing the lowest concentration range that inhibited bacterial growth (Table 2). The MIC of Hexidine™ was found between 3.90 µg/ml and 7.81 µg/ml representing the lowest concentration range that inhibited bacterial growth. Concentrations higher than 31.25 µg/ml showed complete inhibition for Turmwash™ and Concentrations higher than 3.90 µg/ml showed complete inhibition for Hexidine™. Absorbance readings confirmed the gradual increase in bacterial growth with decreasing concentrations. Hexidine™ with concentration of 7.81 µg/ml is more effective than Turmwash™ with concentration of 31.25 µg/ml). These findings suggest that Turmwash™ has a higher MIC than Hexidine™,

which means it requires a greater concentration to inhibit bacterial growth.

Table 1: The ZOI measurements for Turmwash and Hexidine.

Sample	Turmwash (mm)	Hexidine (mm)
1	20	24
2	21	24
3	20	25
4	22	24

Table 2: The MIC measurements.

Concentration (µg/ml)	Absorbance at 600 nm	Significant microbial growth of Turmwash	Significant microbial growth of Hexidine
31.25 A6	2.049	-	-
15.62 A7	1.56	+	-
7.81 A8	1.26	+	-
3.90 A9	1.25	+	+
1.95 A10	1.216	+	+

‘+’ indicates bacterial growth, ‘-’ indicates no bacterial growth

DISCUSSION

A mouthwash is a therapeutic liquid held and swished in the oral cavity to help reduce microbial load through the mechanical action of the oral muscles. Historically, mouthrinses have been used for centuries, with early formulations rooted in traditional practices. Hippocrates recommended a blend of salt, alum, and vinegar, while ancient Talmudic texts suggested rinsing with “dough water” mixed with olive oil. The Greek physician Pedanius Dioscorides later devised more complex preparations using ingredients such as olive leaf decoctions, milk, wine, pomegranate peel, nutgalls, and vinegar. These early mixtures highlight how ancient cultures relied on natural substances to create primitive yet functional mouthwashes.⁸

Chlorhexidine is widely recognized for its effectiveness against oral pathogens, yet its limitations necessitate alternative approaches. The present study demonstrates that Turmwash, a herbal mouthwash, exhibits antibacterial activity comparable to that of chlorhexidine against *A. actinomycetemcomitans*. Vinod et al in the year 2018 compared the antimicrobial efficacy of 0.12% chlorhexidine and new formulated herbal mouthwash after using for 14 days and their study concluded that herbal mouthwash formulation performed effectively well on long term usage and could be used as an alternative mouthwash to overcome the disadvantages of chlorhexidine.⁹ In vitro studies have shown that certain herbal extracts, such as *Eucalyptus camaldulensis*, can exhibit antibacterial activity comparable to or even exceeding that of 0.2% chlorhexidine against oral pathogens like *Streptococcus mutans*. Rahmandost et al demonstrated that *E. camaldulensis* significantly reduced

bacterial colony counts, suggesting that specific phytochemicals within the extract may disrupt bacterial membranes or inhibit biofilm formation.¹⁰ These findings highlight the potential of carefully formulated herbal mouthwashes as effective alternatives to chlorhexidine, particularly for long-term oral hygiene where minimizing side effects is a priority.

The agar diffusion results revealed no statistically significant difference between the two formulations. Although Hexidine displayed marginally greater inhibition zones, Turmwash produced consistent antibacterial effects, reflecting its potential effectiveness. Several recent in vitro studies support that some herbal based mouthwashes or extracts can show antibacterial efficacy comparable to 0.2% CHX, at least against common oral pathogens like *S. mutans*, *Lactobacillus*, *P. gingivalis*, etc.¹¹ The MIC findings further supported these results, with Turmwash inhibiting growth at concentrations as low as 15.62–31.25 µg/ml. This suggests that the phytochemical constituents of Turmwash-likely curcuminoids and essential oils-possess robust antimicrobial properties known to disrupt bacterial membranes, inhibit protein synthesis, and suppress biofilm formation.

In this study, the test mouthwash that is Turmwash's main ingredients are turmeric, clove oil, herbal ingredients have proven to have great antibacterial and antimicrobial properties. Curcumin is a natural compound with several beneficial properties, especially for oral health. It has strong anti-inflammatory effects, helping reduce gum inflammation and bleeding associated with periodontal disease. Its antimicrobial activity inhibits the growth of harmful oral bacteria and prevents biofilm formation, supporting oral hygiene. Curcumin also promotes healing by aiding gum tissue repair and improving clinical outcomes when used alongside standard treatments like scaling and root planning. Additionally, it acts as a potent antioxidant, protecting tissues from oxidative damage, and is generally safe and well-tolerated, making it a promising natural adjunct for maintaining oral and overall health.¹²

Xu et al in 2018 published an extensive review on curcumin's bioactivity and health benefits, highlighting its strong anti-inflammatory, antioxidant, antimicrobial, and anticancer properties.¹³ Given the adverse effects associated with prolonged chlorhexidine use, including staining and mucosal irritation, herbal mouthwashes offer an attractive alternative for long-term oral hygiene regimens. Their natural composition, minimal toxicity, and broad therapeutic benefits make them suitable for individuals seeking holistic and sustainable oral care solutions.

Limitations

The present study is limited by its in vitro design, which does not fully capture the complexity of in vivo oral

conditions. Therefore, further in vivo investigations are warranted to validate these findings and to support the potential role of herbal products in achieving and maintaining optimal oral hygiene.

CONCLUSION

The herbal mouthwash Turmwash demonstrated substantial antibacterial activity against *A. actinomycetemcomitans*, comparable to that of chlorhexidine. Although Hexidine showed slightly higher ZOI values, the difference was not statistically significant. The MIC findings further support the efficacy of the herbal formulation. Turmwash may serve as a viable, natural alternative to chlorhexidine, offering effective antibacterial action with fewer adverse effects.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

- Nagappan N, Palaneeswaran K, Kumarappan K, Natarajan R, Tajuddin R, Anusha Y. Antimicrobial efficacy of herbal and chlorhexidine mouthrinse against *Staphylococcus aureus*-An in vitro microbiological study. J Pharm Bioall Sci. 2022;14:318-22.
- Akbar P, Salahuddin N, Ahmad Z, Shah SZ, Shah F, Maknoon D. Role of Actinomyces species in oral biofilm formation and dental plaque-related diseases. Innov Res Appl Biol Chem Sci. 2024;2(1):120-5.
- Richa G, Pudukalkatti PS, Joshi V. Evaluation and comparison of the antimicrobial effect of two different mouthwashes on selected periodontal pathogens: An in vitro study. J Curr Res Sci Med. 2017;3:40-4.
- Tang G, Yip HK, Samaranayake LP, Luo G, Lo ECM, Teo CS. Actinomyces spp. in supragingival plaque of ethnic Chinese preschool children with and without active dental caries. Caries Res. 2003;37(5):381-90.
- Deus FP, Ouanounou A. Chlorhexidine in dentistry: pharmacology, uses, and adverse effects. Int Dent J. 2022;72(3):269–77.
- Pathan MM, Bhat KG, Joshi VM. Comparative evaluation of the efficacy of a herbal mouthwash and chlorhexidine mouthwash on select periodontal pathogens: An in vitro and ex vivo study. J Indian Soc Periodontol. 2017;21:270-5.
- Verma M, Khan MA, Pathak AK, Lal N, Garg R. Magic of Ayurveda in the treatment of pyorrhea-a review article. World J Pharma Med Res. 2023;9(2):75-87.
- Pagare IS, Beldar S, Mandage UR, Shirsath RB, Patil JR. Herbal mouthwash: a detailed review and analysis. Int J Res Anal Rev. 2024;11(4):178.

9. Vinod KS, Sunil KS, Sethi P, Bandla RC, Singh S, Patel D. A novel herbal formulation versus chlorhexidine mouthwash in efficacy against oral microflora. J Int Soc Prevent Communit Dent. 2018;8:184-90.
10. Rahmandost M, Babaei A, Momeni Z. Antibacterial effects of Zataria multiflora and Eucalyptus camaldulensis extracts versus 0.2% chlorhexidine on Streptococcus mutans count: an in vitro study. BMC Complement Med Ther. 2025;25:413.
11. Fischman SL. The history of oral hygiene products: How far have we come in 6000 years. Periodontol. 2000;15:7-14.
12. Singh S, Aggarwal BB. Curcumin: the Indian solid gold. J Periodontol. 2015;86(10):1320–6.
13. Xu XY, Meng X, Li S, Gan RY, Li Y, Li HB. Bioactivity, health benefits, and related molecular mechanisms of curcumin: current progress, challenges, and perspectives. Nutrients. 2018;10(10):1553.

Cite this article as: Pradhan LR, Chaudhry K, Khanduri N, Gupta Y, Dhyani C. Comparative antibacterial efficacy of a commercially available herbal mouthwash and chlorhexidine mouthwash against *Aggregatibacter actinomycetemcomitans*: an *in vitro* study. Int J Contemp Pediatr 2026;13:405-9.