Benites-Flores C, et al. TUMI Oral Health. 2026;1(2): e004
https://doi.org/10.64716/4n8c6d42

TUMI Oral health

ORIGINAL RESEARCH **Open Access**

Antibacterial effect of hydroethanolic extracts of *Mentha piperita* and *Cymbopogon citratus* against *Streptococcus mutans* ATCC 25175: In vitro study.

Carlos Edilberto Benites-Flores [1], Rossemarie Jetzabel Loaiza-Molina [2]

[1]Catholic University of Los Angeles of Chimbote, Undergraduate, Trujillo, Peru.

[2]Universidade Cidade de São Paulo, São Paulo, Brasil.

Corresponding author

Carlos Edilberto Benites-Flores
e-mail: carlosbenantg@mail.com
Catholic University of Los Angeles of Chimbote, Undergraduate, Trujillo, Peru.

Received: April 16, 2026
Revised: April 18, 2026
Accepted: April 28, 2026

Academic Editor
Yovana Gutierrez, MSc

Abstract

Dental caries remains one of the most prevalent oral diseases worldwide, primarily associated with the activity of Streptococcus mutans. The search for natural antimicrobial agents has gained increasing attention due to their potential effectiveness and reduced adverse effects compared to conventional agents. This study aimed to evaluate the antibacterial effect of hydroethanolic extracts of Mentha piperita and Cymbopogon citratus, individually and in combination, against Streptococcus mutans ATCC 25175. An in vitro experimental, prospective, and analytical study was conducted using 50 Petri dishes distributed into five groups: hydroethanolic extract of Mentha piperita, Cymbopogon citratus, their combination, 0.12% chlorhexidine gluconate (positive control), and a negative control. All groups were exposed to a standardized bacterial suspension of 1.5×10^8 CFU/mL. Antibacterial activity was assessed by measuring inhibition zones using a calibrated Vernier caliper. The combination of extracts at 100% concentration showed the highest antibacterial effect with a mean inhibition zone of 18.8 mm, while individual extracts of Cymbopogon citratus and Mentha piperita both showed inhibition zones of 16.1 mm. Statistical analysis using ANOVA revealed significant differences among groups ($p < 0.05$). The findings suggest that the combined use of Mentha piperita and Cymbopogon citratus hydroethanolic extracts exhibits enhanced antibacterial activity against Streptococcus mutans, indicating their potential as alternative agents for the prevention of dental caries.

Keywords: Mentha piperita, Cymbopogon citratus, Streptococcus mutans, Antibacterial Agents, Plant Extracts, Dental Caries.

TUMI ORAL HEALTH
ISSN (Online): 3119-7701

Introduction

Dental caries remains one of the most prevalent oral diseases worldwide and continues to represent a major public health burden despite being largely preventable. Contemporary evidence describes caries as a biofilm-mediated, diet-modulated, noncommunicable disease driven by ecological dysbiosis, in which prolonged exposure to fermentable carbohydrates promotes acid production, demineralization of dental tissues, and progressive structural damage. Although caries is now understood as a polymicrobial process, Streptococcus mutans remains one of the most relevant cariogenic species because of its ability to adhere to tooth surfaces, synthesize extracellular polysaccharides, tolerate acidic environments, and contribute to the establishment and persistence of cariogenic biofilms. [1–4]

Chemical plaque control remains an important adjunct to mechanical oral hygiene, and chlorhexidine is still widely used because of its broad antimicrobial activity. However, its long-term use has been associated with undesirable effects, particularly tooth staining, taste disturbance, and mucosal intolerance, which has stimulated interest in safer plant-derived alternatives for oral applications. [5,6] In this context, medicinal plants and their derivatives have shown promising antimicrobial and antibiofilm properties against oral pathogens, including S. mutans. Systematic and narrative reviews have highlighted the growing relevance of herbal products as sources of bioactive compounds for oral biofilm control.[7–9] Among these, Mentha piperita has demonstrated inhibitory activity against oral pathogens, including S. mutans, in both conventional extract-based assays and nanostructured delivery systems.[10–12] Likewise, Cymbopogon citratus has shown antibacterial, anti-adherence, and antibiofilm effects against cariogenic microorganisms in mono-species and polymicrobial models, with some studies also suggesting low cytotoxicity and potential compatibility with oral applications.[13–16]

Despite these promising findings, the currently available evidence still presents important limitations. First, much of the literature has evaluated Mentha piperita and Cymbopogon citratus separately rather than in combination. Second, the reported effects vary according to the type of preparation used, since essential oils, crude extracts, hydroethanolic extracts, and nanoformulations may differ substantially in phytochemical composition, diffusion capacity, and antimicrobial performance. Third, many published studies have focused on antibiofilm behavior, cytotoxicity, or combination with chlorhexidine, whereas fewer investigations have specifically assessed the antibacterial performance of combined hydroethanolic extracts against standardized S. mutans strains under comparable in vitro conditions.[10–16] Therefore, there remains a methodological and translational gap regarding whether combining hydroethanolic extracts of Mentha piperita and Cymbopogon citratus may enhance antibacterial activity against S. mutans beyond the effect of each extract alone.

The objective of this study was to evaluate the antibacterial effect of hydroethanolic extracts of Mentha piperita and Cymbopogon citratus, individually and in combination, against Streptococcus mutans ATCC 25175. This research is justified by its contribution to the scientific understanding of plant-based antibacterial strategies directed at cariogenic microorganisms, as well as by its potential clinical relevance in the search for alternative adjuncts for oral biofilm control and dental caries prevention. [5,7,9]

Methods

Study Design

This was a laboratory-based in vitro experimental analytical study conducted to evaluate the antibacterial effect of hydroethanolic extracts of Mentha piperita and Cymbopogon citratus, individually and in combination, against Streptococcus mutans ATCC 25175. The study followed a parallel-group design with post-intervention assessment of antibacterial activity.

Because this was an in vitro experimental study, the reporting guidelines recommended for observational studies, clinical trials, systematic reviews, case reports, diagnostic accuracy studies, and qualitative research were not applicable.

Experimental units, sample size, and group allocation

The experimental units were Petri dishes inoculated with a standardized suspension of Streptococcus mutans ATCC 25175. Only plates showing adequate bacterial growth under standardized laboratory conditions were included. Plates showing contamination or any visible alteration during the experimental process were excluded.

The sample consisted of 50 Petri dishes, distributed into five groups with 10 replicates per group: combined hydroethanolic extracts of Mentha piperita and Cymbopogon citratus at 50%, 75%, and 100%; hydroethanolic extract of Cymbopogon citratus at 50%, 75%, and 100%; hydroethanolic extract of Mentha piperita at 50%, 75%, and 100%; 0.12% chlorhexidine gluconate as positive control; and sterile physiological saline solution as negative control. The number of replicates was established according to the sample size calculation described in the study protocol, resulting in 10 repetitions per condition.

Preparation of plant extracts

Fresh leaves of Mentha piperita and Cymbopogon citratus were washed with water, disinfected with 70% alcohol, and dried in an oven at 50 °C for 4 hours. The plant material was then manually ground and transferred into amber glass containers at a proportion of 250 g of crushed material per 1000 mL of absolute ethanol. The mixtures were macerated for 7 days with daily agitation. Subsequently, each extract was filtered sequentially through Whatman No. 3, No. 2, and No. 1 filter papers.

The filtrate was concentrated using a rotary evaporator to remove the solvent and obtain the hydroethanolic extract. The final preparations were then adjusted to the concentrations evaluated in the study: 50%, 75%, and 100%.

Bacterial strain and inoculum standardization

A pure culture of Streptococcus mutans ATCC 25175 was prepared under microbiological supervision and reactivated on 5% sheep blood agar under anaerobic conditions at 37 °C using a GasPak system. For strain preservation, Mitis Salivarius agar was used. Inoculum standardization was performed from an 18-hour culture by selecting isolated colonies of similar morphology and suspending them in sterile distilled water. Turbidity was adjusted to 0.5 McFarland, equivalent to approximately 1.5 × 10^8 CFU/mL, and verified spectrophotometrically at 625 nm. The standardized inoculum was used within 15 minutes of preparation.

Antibacterial testing procedure

Antibacterial activity was evaluated using the agar disk diffusion method, a commonly used in vitro approach for screening antimicrobial activity. Broth dilution methods are useful for determining inhibitory concentrations, whereas diffusion-based techniques allow direct comparison of inhibition zones under standardized conditions. [17]

Briefly, standardized suspensions of S. mutans ATCC 25175 were inoculated onto culture plates prepared according to the study protocol. The corresponding test substances were then applied to each experimental condition: combined hydroethanolic extracts of Mentha piperita and Cymbopogon citratus at 50%, 75%, and 100%; hydroethanolic extract of Cymbopogon citratus at 50%, 75%, and 100%; hydroethanolic extract of Mentha piperita at 50%, 75%, and 100%; 0.12% chlorhexidine gluconate as positive control; and sterile physiological saline solution as negative control.

All plates were incubated under anaerobic conditions at 36–37 °C for 24 hours. After incubation, the antibacterial effect was determined by measuring the diameter of the inhibition halo in millimeters.

Measurement instrument

The primary outcome was the diameter of the inhibition halo (mm). Measurements were obtained using a calibrated Vernier caliper and recorded in a structured data collection form designed for the study. Because the outcome was a direct physical measurement, formal psychometric validity and reliability testing were not applicable. However, the instrument was calibrated before data collection, and all measurements were performed by a single calibrated examiner to ensure consistency.

Variables

The independent variables were the hydroethanolic extracts of Mentha piperita and Cymbopogon citratus, evaluated individually and in combination at different concentrations (50%, 75%, and 100%). The dependent variable was the antibacterial effect against Streptococcus mutans ATCC 25175, operationalized as the diameter of the inhibition halo measured in millimeters.

Statistical Analysis

Data were entered and analyzed using IBM SPSS Statistics version 26.0. Descriptive statistics were used to summarize the inhibition halo values for each group, including mean and standard deviation. Inferential analysis was performed using the Kruskal–Wallis test, according to the final analytical approach applied in the study tables. Statistical significance was established at $p < 0.05$.

Ethical Considerations

This study was conducted in accordance with the institutional principles of scientific integrity and with the ethical principles of the Declaration of Helsinki [18]. Since this was an in vitro study involving bacterial strains and plant extracts only, with no human participants, no identifiable human data, and no collection of biological samples from patients, informed consent was not required. The protocol was reviewed and authorized by the corresponding institutional academic and research authorities in accordance with the regulations in force at the time of the study.

Results

A statistically significant antibacterial effect was observed across all evaluated extracts against Streptococcus mutans ATCC 25175 ($p < 0.001$). The combination of Mentha piperita and Cymbopogon citratus exhibited a concentration-dependent increase in inhibition zones, with mean diameters rising from 10.0 mm at 50% to 18.8 mm at 100% (Table 1).

Similarly, Cymbopogon citratus alone demonstrated increased antibacterial activity with concentration, reaching a maximum mean inhibition zone of 16.8 mm at 100% (Table 2), while Mentha piperita showed a comparable trend, with inhibition increasing from 11.0 mm at 50% to 16.4 mm at 100% (Table 3). No antibacterial activity was observed in the negative control, whereas the positive control (chlorhexidine 0.12%) consistently showed the highest inhibition across all groups. These findings indicate a clear dose-response relationship, with the combined extract demonstrating greater antibacterial activity compared to individual extracts at equivalent concentrations.

Table 1. Antibacterial effect of hydroethanolic extract combination of Mentha piperita and Cymbopogon citratus against Streptococcus mutans ATCC 25175.

Extracts	n	Mean (mm)	SD	p-value*
Mentha piperita + Cymbopogon citratus (50%)	10	10.0	0.33	
Mentha piperita + Cymbopogon citratus (75%)	10	14.9	0.36	
Mentha piperita + Cymbopogon citratus (100%)	10	18.8	0.43	<0.001
Negative control (saline solution)	10	0.00	0.00	
Positive control (chlorhexidine 0.12%)	10	20.2	0.67	

Diameter of inhibition zone expressed in millimeters (mm)
*Kruskal–Wallis test ($p < 0.05$)

Table 2. Antibacterial effect of hydroethanolic extract of Cymbopogon citratus against Streptococcus mutans ATCC 25175.

Extracts	n	Mean (mm)	SD	p-value*
Cymbopogon citratus (50%)	10	8.0	0.13	
Cymbopogon citratus (75%)	10	14.0	0.38	
Cymbopogon citratus (100%)	10	16.8	0.42	<0.001
Negative control (saline solution)	10	0.00	0.00	
Positive control (chlorhexidine 0.12%)	10	19.3	0.72	

Diameter of inhibition zone expressed in millimeters (mm)
*Kruskal–Wallis test ($p < 0.05$)

Table 3. Antibacterial effect of hydroethanolic extract of Mentha piperita against Streptococcus mutans ATCC 25175.

Extracts	n	Mean (mm)	SD	p-value*
Mentha piperita (50%)	10	11.0	0.13	
Mentha piperita (75%)	10	13.9	0.37	
Mentha piperita (100%)	10	16.4	0.40	<0.001
Negative control (saline solution)	10	0.00	0.00	
Positive control (chlorhexidine 0.12%)	10	18.6	0.71	

Diameter of inhibition zone expressed in millimeters (mm)
*Kruskal–Wallis test ($p < 0.05$)

Discussion

The present study demonstrated that hydroethanolic extracts of Mentha piperita and Cymbopogon citratus, particularly when used in combination, exhibit a concentration-dependent antibacterial effect against Streptococcus mutans ATCC 25175. The highest inhibitory activity observed at 100% concentration suggests a potentiation effect between both extracts. This finding is consistent with the current understanding that plant-derived compounds can enhance antimicrobial activity when combined, particularly against biofilm-forming microorganisms such as S. mutans, which plays a central role in dental caries development [3].

Previous evidence supports the antibacterial and antibiofilm activity of plant extracts against oral pathogens. Milho et al. [8] reported that several medicinal plants, including Cymbopogon citratus, exhibit strong inhibitory effects against oral biofilms, particularly those associated with S. mutans. Similarly, Oliveira et al. [14] demonstrated that C. citratus significantly reduces polymicrobial biofilm formation with low cytotoxicity, reinforcing its potential use in oral health applications. These findings are consistent with the results of the present study, where increasing concentrations of C. citratus led to greater antibacterial activity.

Regarding Mentha piperita, its antibacterial properties have also been widely documented. Ashrafi et al. [11] reported that peppermint-derived compounds significantly inhibit biofilm formation and bacterial adhesion in S. mutans, especially when incorporated into delivery systems such as nanogels. Likewise, Mostafa et al. [12] found that essential oils from Mentha species exhibit strong antimicrobial activity, with inhibition patterns comparable to conventional agents. These findings align with the present results, where Mentha piperita showed increased antibacterial activity as concentration increased.

The enhanced antibacterial effect observed in the combination of both extracts may be explained by the interaction of their phytochemical components. Compounds such as menthol, menthone, and flavonoids from Mentha piperita, together with citral and other terpenoids from Cymbopogon citratus, have been shown to disrupt bacterial cell membranes, alter permeability, and interfere with enzymatic activity [13,21]. Furthermore, previous studies have demonstrated that essential oil components may act synergistically, enhancing antimicrobial efficacy when combined [20,22]. This synergistic behavior likely explains the higher

inhibition observed in the combined extracts compared to individual treatments.

From a clinical perspective, these findings suggest that plant-derived antimicrobial agents may represent a promising alternative or adjunct to conventional treatments such as chlorhexidine, which, although effective, is associated with adverse effects during prolonged use [5]. The use of natural extracts could contribute to safer and more sustainable strategies for controlling cariogenic bacteria and preventing dental caries.

Despite these promising findings, several limitations should be considered. This study was conducted under in vitro conditions, which do not fully replicate the complexity of the oral environment, including saliva, host response, and multispecies biofilms. Additionally, cytotoxicity and long-term safety of the extracts were not evaluated. Future research should focus on assessing these extracts in multispecies biofilm models, evaluating their biocompatibility, and conducting in vivo or clinical studies to confirm their effectiveness under real oral conditions.

Conclusions

The hydroethanolic extracts of Mentha piperita and Cymbopogon citratus demonstrated a concentration-dependent antibacterial effect against Streptococcus mutans ATCC 25175. The combined extracts showed greater inhibitory activity compared to the individual extracts, with the highest effect observed at 100% concentration. Both plant extracts exhibited measurable antibacterial activity, although lower than the positive control.

Author Contributions Statement (CRediT)

BFCE: Conceptualization, Methodology, Writing – Original Draft, Supervision.

BFCE: Formal analysis, Data curation, Validation, Visualization.

BFCE: Resources, Project administration, Funding acquisition, Writing – Review & Editing.

Acknowledgments and Funding

The authors would like to acknowledge the academic authorities and faculty members of the dental program for their support in facilitating data collection, as well as the students who voluntarily participated in this study.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no financial, institutional, or personal conflicts of interest that could have influenced the conduct or publication of this study.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

[1] GBD 2021 Oral Disorders Collaborators. Trends in the global, regional, and national burden of oral conditions from 1990 to 2021: a systematic analysis for the Global Burden of Disease Study 2021. Lancet. 2025;405(10482):897-910. https://doi.org/10.1016/S0140-6736(24)02811-3

[2] Ribeiro AA, Paster BJ. Dental caries and their microbiomes in children: what do we do now? J Oral Microbiol. 2023;15(1):2198433. https://doi.org/10.1080/20002297.2023.2198433

[3] Gao Z, Chen X, Wang C, Song J, Xu J, Liu X, et al. New strategies and mechanisms for targeting Streptococcus mutans biofilm formation to prevent dental caries: A review. Microbiol Res. 2024;278:127526. https://doi.org/10.1016/j.micres.2023.127526

[4] Zhu Y, Wang Y, Zhang C, Li M, Li Y, Ying X, et al. Association of polymicrobial interactions with dental caries development and prevention. Front Microbiol. 2023;14:1196619. https://doi.org/10.3389/fmicb.2023.1162380

[5] Deus FP, Ouanounou A. Chlorhexidine in dentistry: Pharmacology, uses, and adverse effects. Int Dent J. 2022;72(3):269-277. https://doi.org/10.1016/j.identj.2022.01.005

[6] James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. 2017;(3):CD008676. https://doi.org/10.1002/14651858.CD008676.pub2

[7] Karygianni L, Al-Ahmad A, Argyropoulou A, Hellwig E, Anderson AC, Skaltsounis AL. Natural antimicrobials and oral microorganisms: A systematic review on herbal interventions for the eradication of multispecies oral biofilms. Front Microbiol. 2016;6:1529. https://doi.org/10.3389/fmicb.2015.01529
[8] Milho C, Silva J, Guimarães R, Ferreira ICFR, Barros L, Alves MJ. Antimicrobials from medicinal plants: An emergent strategy to control oral biofilms. Appl Sci. 2021;11(9):4020. https://doi.org/10.3390/app11094020
[9] Cardoso VFdS, Roppa RHA, Antunes C, Moraes ANS, Santi L, Konrath EL. Efficacy of medicinal plant extracts as dental and periodontal antibiofilm agents: A systematic review of randomized clinical trials. J Ethnopharmacol. 2021;281:114541. https://doi.org/10.1016/j.jep.2021.114541
[10] Madhavan SS, Sreedevi PV, Varghese M, Joseph A, et al. Effectiveness of Mentha piperita leaf extracts against oral pathogens: An in vitro study. J Contemp Dent Pract. 2018;19(9):1042-1046. https://doi.org/10.5005/jp-journals-10024-2378
[11] Ashrafi B, Rashidipour M, Marzban A, Soroush S, Azadpour M, Delfani S, et al. Mentha piperita essential oils loaded in a chitosan nanogel with inhibitory effect on biofilm formation against S. mutans on the dental surface. Carbohydr Polym. 2019;212:142-149. https://doi.org/10.1016/j.carbpol.2019.02.018
[12] Mostafa DA, Bayoumi FS, Taher HM, Abdelmonem BH, Eissa TF. Antimicrobial potential of Mentha spp. essential oils as raw and loaded solid lipid nanoparticles against dental caries. Res J Pharm Technol. 2020;13(9):4415-4422. https://doi.org/10.5958/0974-360X.2020.00781.7
[13] Tofiño-Rivera A, Ortega-Cuadros M, Galvis-Pareja D, Jiménez-Rios H, Merini LJ, Martínez-Pabón MC. Effect of Lippia alba and Cymbopogon citratus essential oils on biofilms of Streptococcus mutans and cytotoxicity in CHO cells. J Ethnopharmacol. 2016;194:749-754. https://doi.org/10.1016/j.jep.2016.10.044
[14] Oliveira MAC, Borges AC, Brighenti FL, Salvador MJ, Gontijo AVL, Koga-Ito CY. Cymbopogon citratus essential oil: effect on polymicrobial caries-related biofilm with low cytotoxicity. Braz Oral Res. 2017;31:e89. https://doi.org/10.1590/1807-3107BOR-2017.vol31.0089
[15] Mouta LFGL, Marques RS, Antunes AÁ, de Oliveira Lima E, de Souza EL, Ricomini-Filho AP. Cymbopogon citratus essential oil increases the effect of digluconate chlorhexidine on microcosm biofilms. Pathogens. 2022;11(10):1067. https://doi.org/10.3390/pathogens11101067
[16] Pallavi P, Sahoo PP, Sen SK, Raut S. Comparative evaluation of anti-biofilm and anti-adherence potential of plant extracts against Streptococcus mutans: A therapeutic approach for oral health. Microb Pathog. 2024;188:106514. https://doi.org/10.1016/j.micpath.2023.106514
[17] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-79. https://doi.org/10.1016/j.jpha.2015.11.005
[18] World Medical Association. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-2194. https://doi.org/10.1001/jama.2013.281053
[19] Nostro A, Blanco AR, Cannatelli MA, et al. Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. FEMS Microbiol Lett. 2004;230(2):191–195. https://doi.org/10.1016/S0378-1097(03)00890-5
[20] Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;17(4):3989–4006. https://doi.org/10.3390/molecules17043989
[21] Burt S.Essential oils: their antibacterial properties and potential applications in foods.Int J Food Microbiol. 2004;94(3):223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022
[22] Langeveld WT, Veldhuizen EJA, Burt SA.Synergy between essential oil components and antibiotics.Crit Rev Microbiol. 2014;40(1):76–94. https://doi.org/10.3109/1040841X.2013.763219