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Original Research Article

Comparative *in vitro* antibacterial and antifungal efficacy of chemical, herbal and probiotic mouthrinses against *Streptococcus mutans* and *Candida* species

Jasmin Basheer[1], Jasna M. Jain[1], Jincy James[1], Josna Joseph[1], Malavika Muralidharan[1], Sajmi Sathar[1], Rani S. Jayamohan[1], Ananthu Raj[2], Harish Kumar K. S.[1]*

[1]Department of Medical Microbiology, School of Medical Education, Centre for Professional and Advanced studies, Kottayam, Kerala, India
[2]Department of Microbiology, Mar Baselios Dental College, Kothamangalam, Kerala, India

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***Correspondence:**
Dr. Harish Kumar K. S.,
E-mail: drharishkumarks@gmail.com

ABSTRACT

Background: Dental caries and oral candidiasis are predominant biofilm-associated infections, primarily driven by *Streptococcus mutans* and *Candida* species. While mechanical plaque removal is fundamental, mouthrinses are widely used as adjunctive treatments. This study evaluated and compared the in vitro antibacterial and antifungal efficacy of commercially available chemical, herbal and probiotic mouthrinses against key oral pathogens.
Methods: 15 commercial mouthrinses, comprising five each of chemical, herbal and probiotic formulations were tested against *S. mutans* MTCC 457, *Candida albicans* MTCC 227 and clinical isolates of *C. albicans*, *C. tropicalis* and *Pichia kudriavzevii*. Antibacterial and antifungal activity was assessed using the agar well diffusion method. Zones of inhibition were measured following 24 - 48 hours of incubation at 37°C with positive controls penicillin or fluconazole.
Results: Chemical mouthrinses demonstrated the strongest antimicrobial activity, where Hexidine produced the largest mean ZOI, followed by Colgate maXFresh PLAX. Herbal formulations such as Himalaya active fresh and K. P. Namboodiri's Herbal Fresh showed moderate efficacy. Probiotic mouthrinses displayed limited and selective activity, primarily against *S. mutans* and non-albicans *Candida* spp., with Perfora probiotic rinse demonstrating the highest mean ZOI. Several products in each category produced no measurable ZOI.
Conclusions: Chlorhexidine based mouthrinses remain the gold standard for rapid, broad-spectrum microbial reduction. Herbal formulations provide a moderate, phytochemical dependent alternative with a safer profile, while probiotic mouthrinses offer limited but selective antimicrobial effects.

Keywords: Mouthrinses, Chlorhexidine, Antimicrobial efficacy, Probiotic mouthrinses, Herbal mouthrinses

INTRODUCTION

Dental caries and periodontal diseases remain the most prevalent biofilm-associated infections worldwide, with their global burden escalating as the aging populations retain greater numbers of natural teeth. Oral biofilm presents as polymicrobial communities of bacteria and fungi embedded in a highly specialized extracellular matrix. Biofilm formation is a dynamic and continuous process involving initial reversible attachment, irreversible adhesion, maturation and dispersal.[1] Effective oral self-care practices involving mechanical plaque control is fundamental in reducing biofilm accumulation and controlling dental diseases risks.[2]

Streptococcus mutans, facultatively anaerobic Gram-positive cocci, is commonly found in the human oral cavity and is a primary aetiological agent of dental caries.[3]

It easily colonizes tooth surfaces due of its capability to form biofilms on dental surfaces and through the synthesis of glucans that promote subsequent dental plaque development.[4,5] Oral candidiasis, the most common fungal infection of the oral cavity is caused by *Candida* species, predominantly *Candida albicans*, accounting for more than 80% of lesions. Other species including *Candida glabrata, C. tropicalis, Pichia kudriavzevii* (formerly *C. krusei*), *C. guillermondii, C. lusitaniae, C. parapsilosis, C. pseudotropicalis, and C. stellatoidea* are less frequent but clinically relevant.[6] Oral candidiasis manifests predominantly as acute pseudomembranous candidiasis or oral thrush, although other forms include chronic hyperplastic candidiasis, acute and chronic erythematous candidiasis, median rhomboid glossitis, angular cheilitis and linear gingival erythema.[7] Beyond their individual roles, *S. mutans* and *C. albicans* engage in synergistic interactions such as co-aggregation, that markedly enhance biofilm architecture, acid production and caries progression.[8,9] In addition to providing adhesion sites to each other by co-adhesion, streptococcal lactate serves as carbon source for the yeast proliferation, which in turn reduces the local oxygen tension and provides growth stimulatory factors favouring streptococcal metabolism.[9-11] These interactions can be particularly pronounced in immunocompromised hosts or those receiving corticosteroids, where oral cavity can act as reservoir for systemic dissemination, particularly in apical and marginal periodontitis.[12]

Although mechanical plaque removal remains the cornerstone of oral hygiene, its efficacy is limited in biofilm control, which has sustained the long-standing interest in chemical formulations. Mouthrinses offer the advantage of accessing inaccessible sites and soft and hard oral surfaces that brushing and flossing cannot reach, and significantly reduce the total oral microbial load.[13] Commercial mouthrinses have antimicrobial and breath freshening properties and contain a combination of antiseptics, astringents, breath fresheners, essential oils, flavourings and various other constituents.[14] Conventional chemical agents exhibit potent broad-spectrum activity, but are associated with tooth staining, taste alteration and emerging concerns over microbial resistance with their prolonged use. Herbal extracts provide natural alternatives over chemical agents, while probiotic formulations in which live microorganisms confer health benefits when administered in adequate amounts, have emerged as a promising category.[15] Probiotics lower the oral pH, secrete bacteriocins, competitively inhibit *S. mutans* and *Candida* colonisation, and reduce plaque indices, bleeding on probing and gingivitis.[16-19]

Despite the advances in commercial mouthrinses, in vitro comparative evaluation of the antimicrobial potency of chemical, herbal and probiotic mouthrinses against the key oral pathogens such as *S. mutans* and *Candida* spp. remain scarce. The present study aims at evaluating and comparing the in vitro antimicrobial activities of selected commercially available chemical, herbal and probiotic mouthrinses against *S. mutans* and clinically relevant *Candida* spp.

METHODS

The present study was designed as an in vitro experimental comparative study conducted at the Department of Medical Microbiology, School of Medical Education, Kottayam, Kerala, India, from August 2025 to January 2026. Ethical clearance was obtained from the Institutional Ethics Committee (IEC/27/MICRO/SME-GNR/2025). A total of 15 commercially available mouthrinses were evaluated for their antimicrobial efficacy against *Streptococcus mutans* and *Candida* spp. These products were selected based on their widespread availability in the Kerala region, representation of three major formulation categories (chemical, herbal, and probiotic), and diversity in active ingredients. Mouthrinses commonly available in pharmacies, supermarkets, and online platforms were included to reflect real-world consumer usage, with efforts made to incorporate widely used and representative brands within each category to ensure a balanced comparison.

Microbial strains included *S. mutans* MTCC 457, *Candida albicans* MTCC 227 (IMTECH, Chandigarh, India), and oral clinical isolates of *C. albicans*, *C. tropicalis*, and *Pichia kudriavzevii*. Five mouthrinses each from chemical, herbal, and probiotic categories were evaluated. Chemical mouthrinses included Hexidine Mouthwash (ICPA Health Products Ltd), Listerine Original Mouthwash (Johnson & Johnson Ltd), Betadine Germicide Gargle 2% Mint (G.S. Pharmbutor Pvt. Ltd), DENTE 91 Mouthwash (Enavant Research LLP), and Colgate maXFresh PLAX Mouthwash (Colgate-Palmolive Ltd). Herbal mouthrinses included Nature Sure Aloe Vera Mouthwash (Herbal Health Solutions), K.P. Namboodiri's Herbal Fresh Mouthwash (Ramco Herbals), Dr Dento Mouthwash (HCP Wellness Pvt. Ltd), Apollo Noni Mouthwash (HCP Wellness Pvt. Ltd), and Himalaya Active Fresh Mint Mouthwash (Himalaya Wellness Company). Probiotic mouthrinses included Perfora Probiotic Rinse (HCP Wellness Pvt. Ltd), Balance Alkaline Probiotic Mouthwash (HCP Wellness Pvt. Ltd), Velbiom enKor-D (Unique Biotech Ltd), Dive Gut Guard Oral Care (Vital Wellness Limited), and Healthyr-U Oral Health Probiotic (Advanced Vital Enzymes Pvt. Ltd).

All products were stored at room temperature according to the manufacturers' instructions and tested within their expiry period. Sterility testing of each product was performed by plating on Mueller–Hinton agar prior to antimicrobial efficacy testing.

Agar well diffusion method

Antibacterial activity

S. mutans MTCC 457 was revived by growing in thioglycolate broth at 37°C with 5-10% CO_2 overnight. 0.5 McFarland standardised bacterial suspension was lawn

cultured onto Mueller-Hinton blood agar plates. Wells of diameter 5.5 mm in duplicates were created on the Mueller-Hinton blood agar plates using sterile agar well borer. Each well was filled with 10 µl of respective herbal, chemical or probiotic undiluted mouthrinses. Penicillin was used as the standard antibiotic positive control. After the pre-diffusion, plates were incubated at 37°C with 5-10% CO_2 for 24-48 hours. Post incubation, zones of inhibition were measured, recorded and antibacterial activity was assessed.

Antifungal activity

C. albicans MTCC 227 and clinical strains of *C. albicans, Pichia kudriavzevii and C. tropicalis* was inoculated into Mueller-Hinton broth and incubated at 37°C for 3 hours. The suspension was standardised to 0.5 McFarland turbidity and lawn cultured onto Mueller-Hinton agar supplemented with 2% dextrose and 0.01% methylene blue. Wells were prepared in duplicates and added with 10 µl of each undiluted mouthrinse. Fluconazole served as the standard antifungal positive control. Zone diameters were measured after incubation for 24-48 hours at 37°C and antifungal efficacy was determined.

RESULTS

Antimicrobial activity of chemical mouthrinses

Of the total 5 chemical mouthrinses, only 2 demonstrated measurable antibacterial activity as indicated in the Table 1 and Figure 1. Hexidine produced the largest zone of inhibition against *S. mutans* measuring 16 mm whereas Colgate maXFresh PLAX showed ZOI of 14 mm. Colgate maXFresh PLAX exhibited consistent activity with ZOI of 14 mm against *C. albicans* MTCC 227 and clinical isolates of *C. albicans*, C. tropicalis and *P. kudriavzevii* with zones of inhibition measuring 12, 13 and 16mm respectively. Dente 91, Betadine Germicide Gargle and Listerine Original mouthrinses showed no ZOI in the agar well diffusion assay. Hexidine showed a mean antimicrobial inhibition zone of 18.4 mm, whereas the mean ZOI value of Colgate maXFresh PLAX was found to be 13.8 mm. The mean antibacterial activity of all 5 chemical mouthrinses turned out to be 6 mm.

Their antifungal activity against *C. albicans* MTCC 227 was demonstrated with a mean ZOI value of 7 mm and against the clinical isolates of *C. albicans*, C. tropicalis and *P. kudriavzevii* with 6.2 mm, 7 mm and 6 mm respectively.

Antimicrobial activity of herbal mouthrinses

Two herbal formulations demonstrated antimicrobial activity in the agar well diffusion assay. *S. mutans* was inhibited by K.P. Namboodiri's Herbal Fresh and Himalaya Active Fresh mouthrinses with ZOI values of 16 and 11 mm respectively, with a mean value of 5.4 mm among all the five herbal mouthrinses. Himalaya Active Fresh mouthrinse exhibited antimicrobial activity with mean value of 11.8, the highest among herbal mouthrinses and antifungal activity against both standard and clinical strains of *Candida* spp. K.P. Namboodiri's Herbal Fresh mouthrinse showed inhibition zones of diameter 14 mm against *P. kudriavzevii* and 7 mm against *C. tropicalis* accounting for mean antimicrobial inhibition value of 7.4 mm. Antimicrobial activity was not exhibited by Apollo Noni, Dr Dento and Nature Sure Aloe Vera mouthrinses. The mean ZOI values with herbal mouthrinses against *S. mutans* (5.4 mm), and the standard (3 mm) and clinical strains of *Candida* spp. (2, 5.2 and 3.6 mm) were found to be lower than that of chemical mouthrinses.

Figure 1: Agar well diffusion assay for determination of antimicrobial activity of mouthrinses against (a) *S. mutans* and (b) *Candida* spp.

Antimicrobial activity of probiotic mouthrinses

Antibacterial activity against *S. mutans* was exhibited by Perfora Probiotic Rinse and balance Alkaline Probiotic mouthrinse, measuring 13 mm and 14 mm inhibition zones respectively. Comparatively lower mean ZOI values were seen with probiotic mouthrinses, such as 5mm against *S. mutans*, 2.6 mm and 2.8 mm against *P. kudriavzevii* and C tropicalis respectively. A

nti-*Candida*l activity was not observed in balance Alkaline Probiotic mouthrinse, Velbiom enKor-D and Dive Gut Guard. Healthyr-U inhibited *C. tropicalis* with a mean inhibition zone of 13 mm and a zone diameter of 14 mm was observed against *P. kudriavzevii* by Perfora Probiotic Rinse. Perfora demonstrated the highest antimicrobial activity among the five selected probiotic rinses, with a mean value of 5.4 mm, followed by Healthyr-U (2.6 mm) and Salt Oral Care (2.4 mm).

Antimicrobial activity of mouthrinses with detectable ZOI is demonstrated in Figure 2 and the antimicrobial efficacies of chemical, herbal and probiotic mouthrinses are compared in Figure 3. Figure shows the heat map of antimicrobial efficacy demonstrated by all the tested commercially available mouthrinses.

Table 1: Antimicrobial activity of various chemical, herbal and probiotic mouthrinses.

Strains	Chemical mouthrinses, ZOI (mm)					Mean
	Colgate Plax	Hexidine	Dente91	Betadine	Listerine	
S mutans MTCC 457	14	16	0	0	0	6
Candida albicans MTCC 227	14	21	0	0	0	7
C albicans	12	19	0	0	0	6.2
P. kudriavzevii	16	19	0	0	0	7
C tropicalis	13	17	0	0	0	6
Mean	13.8	18.4	0	0	0	6.44
	Herbal mouthrinses, ZOI (mm)					
Strains	**Himalaya Active Fresh**	**K P Namboodiris**	**Apollo Noni**	**Dr Dento**	**Nature Sure**	**Mean**
S mutans MTCC 457	11	16	0	0	0	5.4
Candida albicans MTCC 227	15	0	0	0	0	3
C albicans	10	0	0	0	0	2
P. kudriavzevii	12	14	0	0	0	5.2
C tropicalis	11	7	0	0	0	3.6
Mean	11.8	7.4	0	0	0	3.84
	Probiotic mouthrinses, ZOI (mm)					
Strains	**Perfora**	**Salt Oral Care**	**Enkor-D**	**Dive Gut Guard**	**Healthyr-U**	**Mean**
S mutans MTCC 457	13	12	0	0	0	5
Candida albicans MTCC 227	0	0	0	0	0	0
C albicans	0	0	0	0	0	0
P. kudriavzevii	14	0	0	0	0	2.8
C tropicalis	0	0	0	0	13	2.6
Mean	5.4	2.4	0	0	2.6	2.08

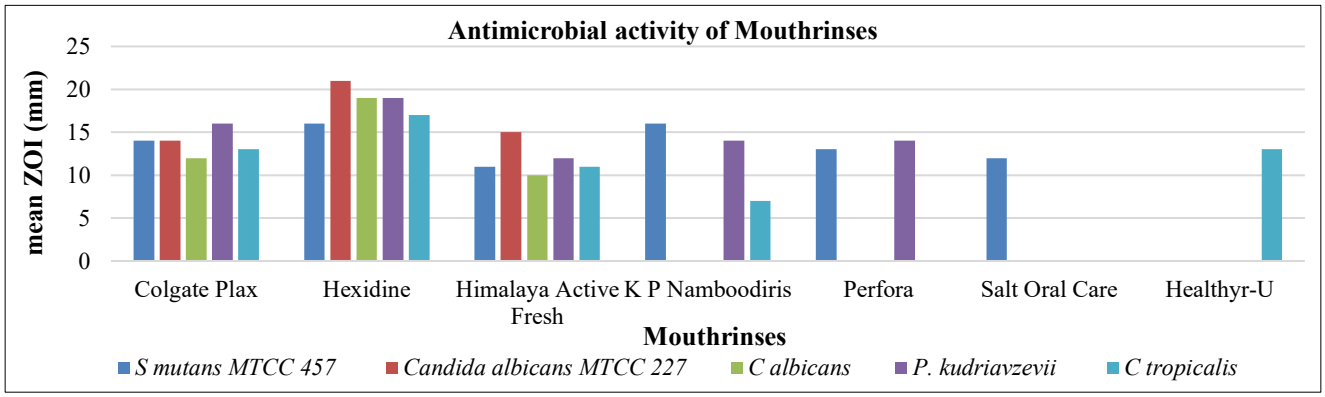

Figure 2: Graph indicating mean ZOI values of chemical, herbal and probiotic mouthrinses with detectable ZOI.

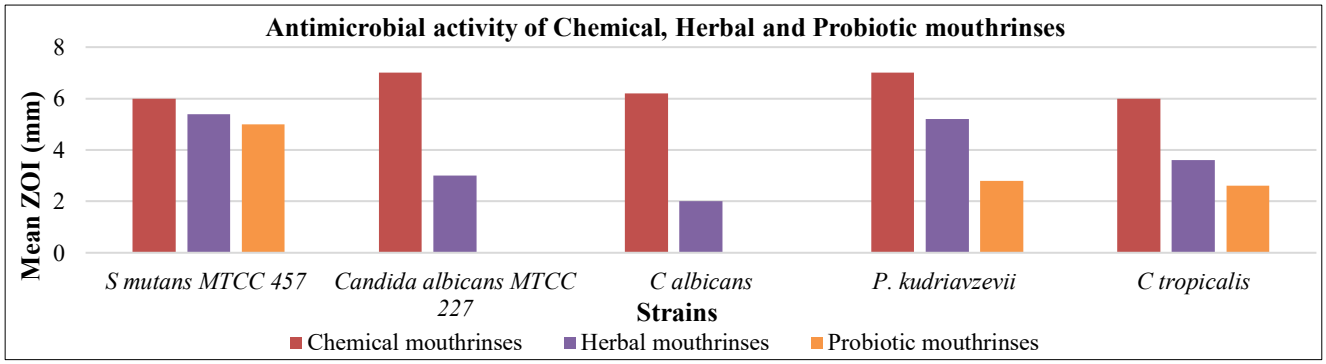

Figure 3: Antibacterial and antifungal activity of chemical, herbal and probiotic mouthrinses.

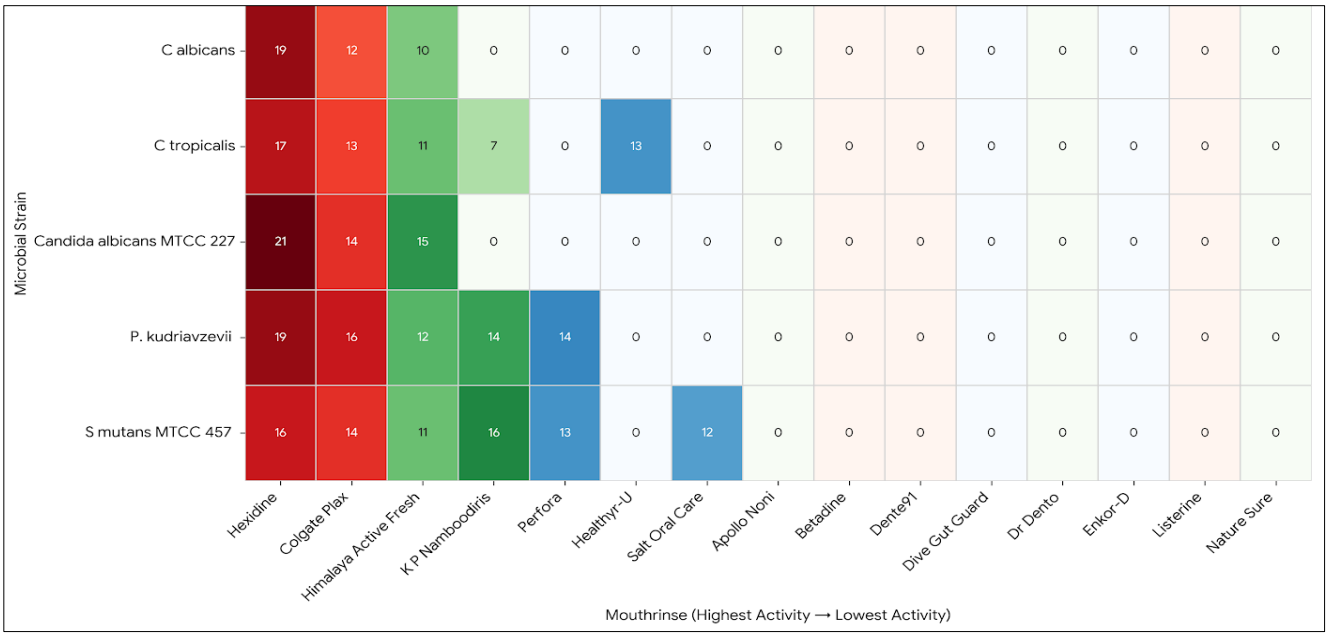

Figure 4: Heat map of antimicrobial efficacy of commercially available chemical, herbal and probiotic mouthrinses.

DISCUSSION

The present investigation gives a comprehensive in vitro evaluation antimicrobial efficacy and comparison of a total of 15 commercially available mouthrinses, including five each of chemical, herbal and probiotic formulations. Agar well diffusion assay revealed elevated efficacy of chemical formulations, particularly with chlorhexidine compared to other mouthrinses, followed by herbal and probiotic formulations. Findings from our study reaffirm chlorhexidine gluconate as the most potent antimicrobial agent among all the tested formulations at the given conditions, showing both antibacterial and antifungal activity. These findings align with previous studies by Parkar et al and Talebi et al, which reported the superior inhibitory effects of Chlorhexidine against oral pathogens, compared to other commercial and herbal formulations.[20,21] The review by Maziere et al further reinforces the broad-spectrum antifungal potential of chlorhexidine, particularly underscoring its efficacy against *Candida* spp., that exhibit resistance to conventional antifungal agents.[22] This study strengthens the evidence supporting chlorhexidine formulations as the gold standard in anti-plaque and anti-gingivitis mouthrinses, due to their extended broad-spectrum antimicrobial and anti-plaque activity.[23] Absence of detectable ZOI in the agar well diffusion assay with the other chemical mouthrinses may be attributed to, the rapid volatilisation of alcohols and essential oils during incubation, suboptimal diffusion of polymers and pH incompatibility with the test media.

The moderate antimicrobial activity of herbal formulations is driven by their rich phytochemical repertoire. These bioactive phytochemicals interfere with bacterial adherence and metabolic processes, inhibiting quorum sensing and biofilm formation, leading to reduced plaque accumulation and prevention of oropathogenic microbial communities.[24] Antimicrobial activity was demonstrated by only two of the five herbal formulations in our study, likely due to the presence of potent phytochemicals and adjunct chemotherapeutic agents. Ezhil and Sakthi reported significant bacterial reduction with the same herbal brands.[25] The limited efficacy observed with the other formulations highlights the critical role of ingredient composition, synergistic interactions and herbal standardisation in phytotherapeutic research for determining antimicrobial efficacy. Mishra et al highlighted that although herbal mouthrinses exhibit lower antimicrobial efficacy compared to chlorhexidine, they offer a more favorable safety profile characterized by reduced adverse effects, rendering them suitable for prolonged use particularly in pediatric or sensitive populations. [26] Probiotic mouthrinses displayed the most limited antimicrobial activity in our study. Perfora Probiotic Rinse and Balance Alkaline Probiotic exhibited modest zones against *S. mutans* and certain *Candida* isolates, while the remaining formulations were inactive, particularly against *C. albicans*. The selective inhibition observed in the current study, primarily against *S. mutans* and non-albicans *Candida* spp., likely reflects the production of bacteriocins, organic acids and competitive metabolites by the specific probiotic strains. Although probiotic mouthrinses demonstrated comparatively lower antimicrobial potency than the other formulations, they could still contribute meaningfully to the maintenance of microbial balance and the prevention of pathogenic recolonization through ecological and immunomodulatory mechanisms.

The demonstration of parallel susceptibility patterns between *S. mutans* and *Candida* spp. to the active agents in our research, may offer benefits in disrupting the

synergistic interactions between these organisms in cariogenic biofilms. Limitations of the current study includes the use of agar well diffusion assay which is not reflective of biofilm resistance, small volumes of test formulations that do not mimic oral rinsing conditions, limited strains and sample size tested. Future studies should employ biofilm models, Minimum inhibitory concentration determinations, saliva simulated conditions, cytotoxicity testing, standardization of herbal formulations and optimization of probiotic strains.

CONCLUSION

This *in vitro* comparative evaluation demonstrates that chlorhexidine-based mouthrinses exhibit the strongest, broadest and consistent antimicrobial activity against *S. mutans* MTCC 457, *C. albicans* MTCC 227 and oral clinical isolates of *C. albicans*, *C. tropicalis* and *P. kudriavzevii*, confirming their position as the gold standard for rapid microbial reduction in the oral cavity. Herbal mouthrinses exhibiting moderate and phytochemical dependent efficacy, offers a promising natural alternative with a favourable safety profile suitable for long term or adjunctive use, especially in populations preferring alcohol free or phytochemical based products. Probiotic mouthrinses with limited but selective inhibitory activity in the study, highlights their potential role in supporting oral microbiota and preventing pathogenic colonization. Findings from the present study may guide the development of next generation mouthrinses providing the synergistic antimicrobial activity of chemical formulations and phytochemicals, along with probiotic mediated microbial balance and patient safety.

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