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COMPARATIVE EVALUATION OF THE ANTIMICROBIAL EFFICACY OF TRIPHALA AND CENTELLA ASIATICA AGAINST CARIOGENIC BIOFILM-FORMING BACTERIA USING MIC TESTING: AN IN VITRO STUDY

Dental Science

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ABSTRACT

Background: Herbal remedies offer natural alternatives to conventional antimicrobials in dentistry. Triphala and Centella asiatica have shown potential against Streptococcus mutans and Lactobacillus casei, two major contributors to dental caries. **Aim:** This study aimed to assess and compare the antimicrobial efficacy of Triphala and Centella asiatica extracts against cariogenic biofilm-forming organisms. **Materials and Methods:** An in vitro experimental study was conducted using MIC assay to determine antibacterial effects.). Chlorhexidine was used as the control. **Results:** Both herbal formulations showed significant antibacterial effects. Triphala displayed greater inhibition of bacterial adhesion and biofilm formation than Centella asiatica, as confirmed by lower MIC values. **Conclusion:** Triphala and Centella asiatica possess notable antimicrobial activity and may serve as effective agents in natural oral hygiene products. Further clinical validation is recommended.

KEYWORDS

Triphala, Centella asiatica, Streptococcus mutans, Lactobacillus casei, Biofilm inhibition, MIC

INTRODUCTION

Herbal medicine has long served as a cornerstone of traditional healthcare systems across various cultures, offering natural and holistic alternatives to synthetic pharmaceuticals. In recent years, there has been renewed interest in plant-based remedies due to their biocompatibility, minimal side effects, and environmental sustainability. Within the realm of oral healthcare, medicinal plants have been employed for centuries to maintain oral hygiene and manage a range of dental infections. Among these, Triphala—a classical polyherbal formulation in Ayurvedic medicine—has attracted considerable attention for its therapeutic efficacy. Comprising three potent fruits—Terminalia chebula, Terminalia bellerica, and Emblica officinalis—Triphala is endowed with a wide spectrum of antimicrobial, antioxidant, and anti-inflammatory properties, positioning it as a promising candidate in the prevention of oral diseases and biofilm control.[1,2]

Triphala has shown particular effectiveness against cariogenic bacteria such as Streptococcus mutans and Lactobacillus casei, which are primary contributors to dental caries and periodontal disorders.[7] T. chebula (Haritaki) contains high levels of tannins, flavonoids, and chebulinic acid, all of which exhibit antibacterial and plaque-inhibiting activities. T. bellerica (Bibhitaki) is rich in gallic acid and ellagic acid, known to reduce bacterial adhesion and plaque formation. E. officinalis (Amla), abundant in vitamin C and antioxidants, enhances host immunity and alleviates oxidative stress in oral tissues.

In addition to Triphala, other herbal agents such as Centella asiatica, Andrographis paniculata, Juglans regia, and Cymbopogon citratus have also demonstrated promising antimicrobial and antibiofilm effects. C. asiatica is valued for its wound-healing properties and ability to inhibit oral biofilms.[1] A. paniculata displays antibacterial activity on par with chlorhexidine. [3] J. regia (Walnut Husk) and C. citratus (Lemongrass) show significant action against S. mutans and Lactobacillus species.[6,7]

Although chlorhexidine remains a gold standard in dental antimicrobial therapy, its long-term use is associated with drawbacks such as tooth staining, altered taste sensation, and resistance development. Growing concerns about antimicrobial resistance underscore the need for safer, plant-based alternatives.[2] This study aims to evaluate and compare the minimum inhibitory concentration of Triphala and Centella asiatica against Streptococcus mutans and Lactobacillus caseii .[1,3,5,7]

MATERIALS AND METHODS

Study Design Samples were divided into two main groups

Group I: Mixed Herbal Powder Extract (MHPE)
- Subgroup A: Untreated (Control)
- Subgroup B: Treated with MHPE

Group II: 0.12% Chlorhexidine Gluconate (CHX)
- Subgroup A: Untreated (Control)
- Subgroup B: Treated with CHX

Methodology

1. Plant Extract Preparation

Powdered extracts of Centella asiatica, Terminalia chebula, Terminalia bellerica, and Emblica officinalis were combined in a 70:10:10:10 ratio. Three solvents were used:
- Methanol (10:1 v/w)
- Chloroform:Methanol (1:1 v/w)
- Chloroform (1:1 v/w)

Extracts were flushed with nitrogen, shaken for 4–5 hours, incubated at 37°C for 24 hours, and pooled. The mixture was concentrated using vacuum evaporation, lyophilized, and stored at –4°C. The final residue was dissolved in 1% DMSO.

2. MIC Evaluation

- Aliquots (200µl, 10 CFU/ml) of S. mutans and L. casei were spread on BHI agar plates.
- Wells were filled with MHPE (1, 10, 25, 50 µg/ml).
- MIC: Lowest concentration showing inhibition zone.

Statistical Analysis

- Data analyzed using SPSS v23.0.
- Shapiro-Wilk test checked data normality.
- As data was normally distributed, parametric tests were used.
- Inferential analysis included Kruskal-Wallis and Mann-Whitney U test.
- Significance level set at $p < 0.05$.

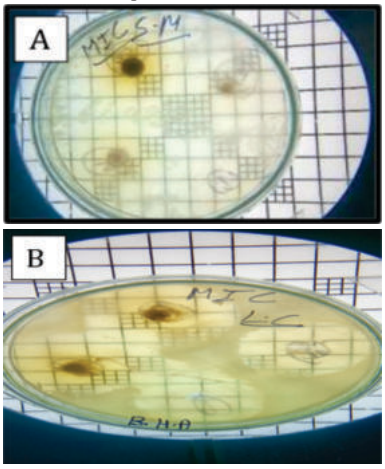


Figure 1: Counting of Bacterial Colonies for MIC (A) Streptococcus Mutans (B) Lactobacillus Casei

RESULTS

This in-vitro study evaluated and compared MIC of Mixed Herbal Powder Extract (MHPE)) against Streptococcus mutans and Lactobacillus casei using the agar diffusion method and scanning electron microscopy (SEM).

Data were compiled using Microsoft Excel and analyzed with SPSS version 21.0. Normality was assessed using the Shapiro-Wilk test. As the data did not follow a normal distribution, non-parametric tests were applied. Mann-Whitney U test was used for group comparisons, with a significance level set at $p < 0.05$.

Table I & Graph 1: Intergroup Comparison of MIC

TABLE I

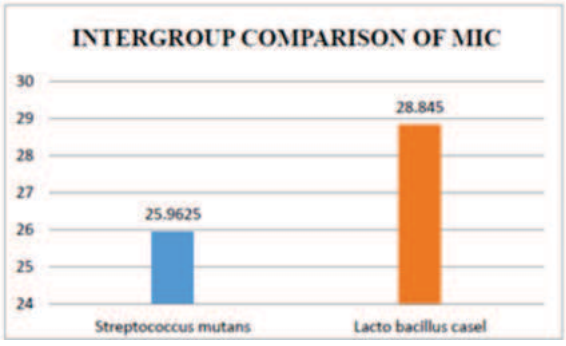
INTERGROUP COMPARATIVE ANALYSIS OF MIC BETWEEN STREPTOCOCCUS AND LACTOBACILLUS GROUPS

Minimum Inhibitory Concentration (MIC)	Mean	Std. Deviation	Std. Error Mean	p-value
Streptococcus mutans	25.9625	.93207	.46604	.012*
Lactobacillus casei	28.8450	1.32067	.66033	

*p<0.05 is considered as statistically significant

GRAPH 1

INTERGROUP COMPARATIVE ANALYSIS OF MIC BETWEEN STREPTOCOCCUS AND LACTOBACILLUS GROUPS.



- The Minimum Inhibitory Concentration (MIC) showed a statistically significant difference between the two bacterial groups ($p = 0.012$).
- S. mutans exhibited a lower MIC (25.96 ± 0.93 µg/ml) compared to L. casei (28.85 ± 1.32 µg/ml), indicating higher sensitivity of S. mutans to MHPE.

DISCUSSION

The present study explores the antimicrobial efficacy of a Mixed Herbal Powder Extract (MHPE) comprising Centella asiatica, Terminalia chebula, Terminalia bellerica, and Emblica officinalis, against Streptococcus mutans and Lactobacillus casei, the primary cariogenic bacteria. MHPE was compared to Chlorhexidine gluconate (CHX), a gold standard in dental antimicrobial treatments.

The rationale behind selecting MHPE stems from its bioactive plant components, known for their synergistic antimicrobial, anti-inflammatory, and antioxidant effects.[8] Previous studies have documented the effectiveness of Centella asiatica and Triphala in inhibiting oral pathogens and disrupting biofilms, thus validating their inclusion in our extract.[9] In this study, MIC analysis showed significant inhibition of S. mutans and L. casei at low concentrations (25.96 ± 0.93 µg/ml and 28.85 ± 1.32 µg/ml, respectively), reflecting the potent antimicrobial nature of MHPE.

Comparing our MIC results with Kolar et al. (2024)[10] who evaluated antimicrobial agents against Gram-positive mastitis pathogens, a similar trend was noted where effective inhibition occurred at or below clinical breakpoints. Conversely, Tomoyama et al. (2023)[11] observed higher MICs for Staphylococcus strains from orthopedic infections, particularly MR-CoNS, indicating the necessity for infection-specific antimicrobial concentrations. These comparisons reinforce the importance of targeted antimicrobial evaluation, as our study demonstrates that oral pathogens may be susceptible to lower concentrations of plant-based agents, unlike systemic or implant-related infections.

When comparing MHPE and CHX, distinct strain-specific effects emerged. MHPE demonstrated significantly greater efficacy against S. mutans biofilms ($p = 0.040$), while CHX was more effective against L. casei ($p = 0.001$).[12,13] This aligns with earlier findings that CHX, although widely used, has limited penetration into dense S. mutans biofilms.[14] SEM imaging confirmed that MHPE disrupted S. mutans biofilm matrices more effectively than CHX, with visible morphological damage and reduction in structural integrity. These findings support the notion that herbal formulations like MHPE can offer targeted antimicrobial activity with enhanced biofilm penetration capabilities.

SEM has proven crucial in visualizing morphological alterations in treated biofilms.[15] In our study, S. mutans biofilms exposed to MHPE exhibited compromised membranes, disorganized clusters, and evident structural degradation. This is consistent with the findings of AlMatar et al. (2022),[16] where silver nanoparticles synthesized from herbal extracts caused substantial biofilm disruption in S. mutans. On the other hand, Kiryakova et al. (2015)[17] did not focus on biofilms but assessed planktonic bacterial sensitivity without SEM analysis, which limits their comparative utility for biofilm-specific studies. Our data reinforce the need to evaluate biofilm resilience, as biofilm-embedded bacteria exhibit higher resistance than planktonic forms.

Against L. casei, MHPE induced moderate morphological changes, including partial cell wall disruption and biofilm disaggregation. While the effect was notable, it was less dramatic than with S. mutans, suggesting a strain-dependent variation in susceptibility.[18] Kamal et al. (2023)[19] also observed inconsistent antimicrobial effects of polyphenol-rich extracts on L. casei, with SEM revealing minor structural damage, likely due to inherent resistance mechanisms. These findings suggest that MHPE may be more effective against specific oral pathogens and supports its consideration for personalized or adjunctive therapies.

CHX treatment, while demonstrating overall efficacy, showed limited disruption of S. mutans biofilm structures. SEM analysis revealed partial biofilm integrity retention, despite membrane damage and cytoplasmic leakage. This corroborates with Mensitieri et al. (2023),[20] who reported strong membrane damage using CHX but acknowledged challenges in complete biofilm penetration. Jiayi et al. (2022)[21] further highlighted reduced susceptibility in certain S. mutans strains, possibly due to prolonged CHX exposure, emphasizing the risk of adaptive resistance.

In contrast, CHX was highly effective against L. casei, with SEM revealing substantial bacterial lysis and biofilm collapse. These results support previous findings by Singh et al. (2023),[22] where SEM showed CHX-induced structural damage in L. casei populations. However, evidence of emerging CHX tolerance, particularly in long-term use scenarios, warrants consideration of alternative treatments such as MHPE, which could reduce dependency on chemical agents.

The biofilm-disrupting potential of MHPE, particularly against S. mutans, is clinically significant. Biofilms confer heightened resistance to antimicrobials, serving as reservoirs for persistent infections and contributing to caries progression. Our findings indicate that MHPE not only possesses antimicrobial properties but can effectively destabilize biofilms, potentially reducing plaque accumulation and disease onset.[23,24] In contrast, CHX's efficacy was restricted by biofilm-mediated shielding, as supported by prior studies showing its limited ability to penetrate thick biofilm matrices.[4]

In summary, MHPE demonstrated potent antimicrobial and antibiofilm properties against S. mutans and moderate effects against L. casei, with greater biofilm penetration compared to CHX. Given the increasing demand for safer, plant-based alternatives in oral healthcare, MHPE emerges as a promising candidate. These findings support the integration of herbal agents into mainstream dental care, particularly for managing biofilm-associated oral pathogens. Future investigations should focus on in vivo validation, formulation stability, and mechanistic insights into MHPE's antimicrobial pathways to substantiate its clinical applicability.

CONCLUSION

This study underscores the significant antimicrobial potential of Mixed Herbal Powder Extract (MHPE), formulated from medicinal plants with known antioxidant, anti-inflammatory, antimicrobial, and immune-boosting properties. MIC and MBC assays confirmed its effectiveness at low concentrations, while SEM analysis revealed substantial structural damage to bacterial biofilms, particularly in Streptococcus mutans and Lactobacillus casei. These findings highlight MHPE's strong bactericidal and biofilm-disrupting capabilities, positioning it as a promising natural alternative to synthetic antimicrobials in dentistry. Its plant-based origin offers added benefits of biocompatibility and reduced risk of side effects, aligning with the growing demand for safer, sustainable oral healthcare solutions. However, further clinical studies and formulation standardization are essential to validate its long-term safety, efficacy, and practical application in routine dental care.

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