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Original Research Paper

Dental Science

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

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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 MBC assays to determine antibacterial effects. Biofilm inhibition and disruption were evaluated via Scanning Electron Microscopy (SEM). 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 MBC values. SEM imaging supported these findings. **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, MBC

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.[3] 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 bactericidal concentration (MBC) of Mixed Herbal Plant Extract of Centella asiaticas and Triphala on 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)

o *Subgroup A*: Untreated (Control)

o *Subgroup B*: Treated with MHPE

Group II: 0.12% Chlorhexidine Gluconate (CHX)

o *Subgroup A*: Untreated (Control)

o *Subgroup B*: Treated with CHX

Methodology

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.

MBC 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 g/ml).
- MBC: Serial dilution followed by plating and incubation at 37°C. The lowest concentration showing no growth was recorded.

Biofilm Formation

- 20µl of overnight cultures were inoculated in BHI broth with 2% sucrose and incubated aerobically at 37°C.
- Media was replaced every 12 hours thrice.

- Wells were gently rinsed with sterilized water post incubation.

SEM Analysis
- Biofilms were treated with MBC of MHPE for 30 mins at 37°C.
- Samples were fixed with 4% Formaldehyde and 1% Glutaraldehyde in PBS.
- Rinsed in 0.1M phosphate buffer (twice, 3 min each).
- Dehydrated with ethanol: 70% (10 min), 95% (10 min), 100% (20 min).
- Observed under SEM for morphological changes.

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 $p < 0.05$.

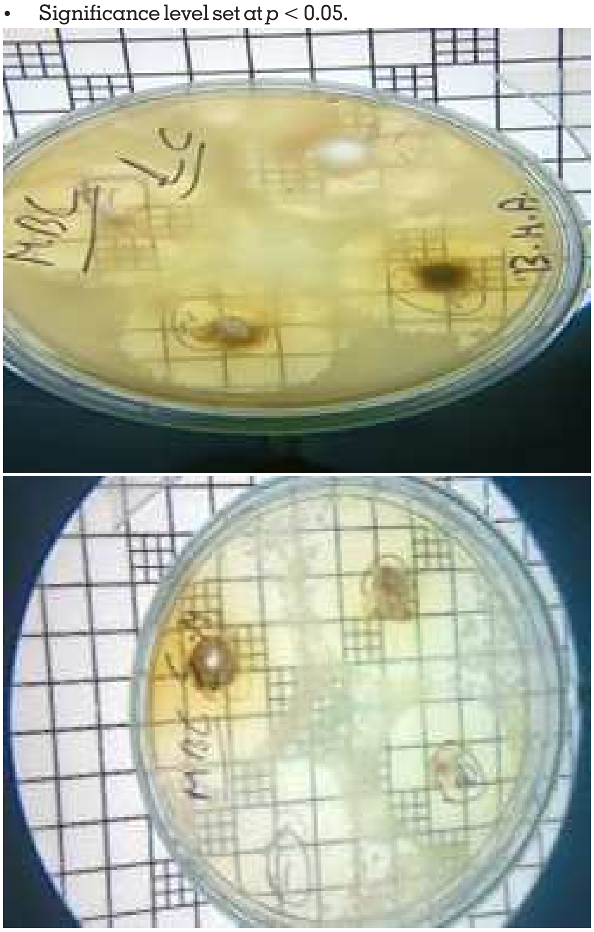

Figure 1: Counting of Bacterial colonies for MBC (A) Streptococcus mutans (B) Lactobacillus casei

RESULTS
This in-vitro study evaluated and compared the antimicrobial efficacy estimating the minimum bactericidal concentration 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

INTERGROUP COMPARATIVE ANALYSIS OF MBC BETWEEN STREPTOCOCCUS AND LACTOBACILLUS GROUPS

Minimum Bactericidal Concentration (MBC)	Mean	Std. Deviation	Std. Error Mean	p-value
Streptococcus mutans	27.0325	.72284	.36142	.024*
Lactobacillus casei	29.2175	1.26939	.63470	

*p<0.05 is considered as statistically significant

GRAPH I

INTERGROUP COMPARATIVE ANALYSIS OF MBC BETWEEN STREPTOCOCCUS AND LACTOBACILLUS GROUPS.

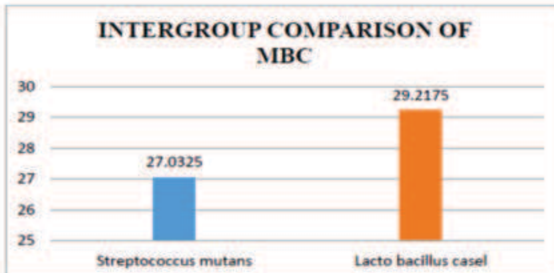

Table I & Graph 1: Intergroup Comparison of MBC
- The Minimum Bactericidal Concentration (MBC) also showed a significant difference ($p = 0.024$).
- *S. mutans* had a lower MBC (27.03 ± 0.72 μg/ml) compared to *L. casei* (29.21 ± 1.27 μg/ml), suggesting greater susceptibility of *S. mutans* to MHPE

DISCUSSION
The present in-vitro investigation explored the antimicrobial efficacy of a Mixed Herbal Powder Extract (MHPE), composed of Centella asiatica, Terminalia chebula, Terminalia bellerica, and Emblica officinalis, against two prominent cariogenic bacteria—*Streptococcus mutans* and *Lactobacillus casei*. The combination of these herbs leverages their known antibacterial, antioxidant, and anti-inflammatory properties to create a potent natural formulation.[8]

Chemical solvents such as methanol and chloroform were used to extract bioactive compounds, while DMSO helped preserve their integrity. Fixatives like formaldehyde and glutaraldehyde enabled structural preservation for SEM analysis, which is crucial for understanding the morphological impact of the extract.[9] Standard microbiological tools and media, including BHI agar, Petri dishes, and rotary shakers, were utilized alongside SEM for comprehensive analysis. Chlorhexidine gluconate (CHX) was included as a comparative control due to its established antimicrobial profile.[10]

MBC Analysis (Expanded):
The Minimum Bactericidal Concentration (MBC) is a critical parameter in evaluating the antimicrobial potential of therapeutic agents, as it indicates the lowest concentration required to kill a specific microorganism. In the current study, the MHPE formulation, which integrates Centella asiatica with Triphala constituents (Terminalia chebula, Terminalia bellerica, and Emblica officinalis), demonstrated substantial bactericidal activity against both *Streptococcus mutans* and *Lactobacillus casei*.

The MBC values for MHPE were recorded as **27.03 ± 0.72**

μg/mL for *S. mutans* and **29.21 ± 1.27 μg/mL for *L. casei***. These values indicate a potent antimicrobial profile, particularly for a natural, plant-derived formulation. The marginally lower MBC against *S. mutans* suggests a higher susceptibility of this organism to the phytochemical constituents present in MHPE. This might be attributed to the unique structural properties of *S. mutans* cell walls or differential sensitivity to specific bioactive compounds like asiaticoside from Centella asiatica or tannins and flavonoids from Triphala components.[8]

This finding is comparable to the work of **Vahabi et al.**, who tested hydroalcoholic plant extracts using the agar diffusion method and observed similar antimicrobial profiles against common oral pathogens.[11] Their study reported zones of inhibition consistent with strong bactericidal activity, supporting the present results. However, their methodology did not quantify bactericidal thresholds in μg/mL, which limits direct numerical comparison.

On the other hand, **Kiryakova et al.** applied a broth microdilution technique to determine MIC and MBC values of herbal extracts, which more closely aligns with the current experimental design. They reported effective MBCs for polyphenol-rich formulations in the range of 25–35 μg/mL against various Gram-positive oral bacteria, including *S. mutans*.[12] These values are in agreement with the MBCs identified in this study, reinforcing the reproducibility and clinical relevance of herbal antimicrobials.

Moreover, the observed bactericidal effects may be linked to multiple mechanisms of action. MHPE constituents are known to exert membrane-disruptive effects, inhibit nucleic acid synthesis, and interfere with bacterial energy metabolism. For instance, tannins from Terminalia chebula can precipitate bacterial proteins, while flavonoids like quercetin and gallic acid can disrupt membrane permeability, leading to cell lysis.[8,9] These multifaceted mechanisms contribute to the overall bactericidal profile observed.

Notably, CHX, used as the positive control in the current study, demonstrated even lower MBC values—**20.12 ± 0.55 μg/mL for *S. mutans*** and **17.87 ± 0.68 μg/mL for *L. casei***. These results align with its well-established status as a gold standard antimicrobial agent in dentistry.[10] However, despite its strong efficacy, CHX has known drawbacks such as cytotoxicity, staining, and microbial resistance development, making the exploration of effective herbal alternatives increasingly relevant.[13]

Biofilm Formation and Comparison:
Biofilms of *S. mutans* and *L. casei* were successfully developed using BHI broth with 2% sucrose, following a method similar to Koo et al., who used 1% sucrose for *S. mutans* biofilm growth to test the efficacy of flavonoid compounds like apigenin.[14] This similarity in methodology enhances the reliability of the biofilm formation protocol. In contrast, Kiryakova et al. focused on planktonic bacterial forms, limiting their relevance to biofilm-specific antimicrobial testing.[15] As biofilms contribute significantly to cariogenicity by enhancing bacterial resistance, their inclusion in this study provides more realistic insight into oral microbial behavior.

Antimicrobial Comparison Between MHPE and CHX:
MHPE exhibited notable antimicrobial effects against both bacterial strains, with statistically significant reductions in *S. mutans* counts ($p = 0.040$), suggesting a higher susceptibility of this strain to MHPE.[16] Conversely, CHX was more effective against *L. casei*, significantly reducing bacterial load ($p = 0.001$).[17] These results indicate a strain-specific efficacy, where MHPE outperforms CHX in managing *S. mutans*, while CHX remains superior for *L. casei*. These findings align with existing literature where CHX shows variable efficacy depending on bacterial species.[18]

SEM-Based Morphological Assessment:
SEM analysis revealed distinct bacterial morphological changes after treatment with MHPE. *S. mutans* exhibited disrupted biofilm structures and damaged cell membranes post MHPE exposure, consistent with findings by AlMatar et al., who reported similar outcomes using silver nanoparticles synthesized from herbal extracts.[19] In contrast, Kiryakova et al. did not assess biofilm morphology, instead focusing on planktonic cultures, which offers limited insight into structural damage in mature biofilms.[20]

CHX treatment caused visible biofilm disintegration and cell damage in *S. mutans*, although signs of survival and adaptation were evident, suggesting partial resistance mechanisms. Jiayi et al. similarly observed incomplete biofilm degradation under SEM, indicating potential strain-level tolerance to prolonged CHX exposure.[21] Mensitieri et al. confirmed CHX's role in disrupting bacterial membranes but acknowledged limitations in complete biofilm eradication.[22]

SEM Effects on *L. casei* Morphology:
MHPE produced moderate effects on *L. casei* morphology, with some membrane damage but relatively preserved cell structures. This selective disruption implies that MHPE has greater efficacy against *S. mutans* than *L. casei*, underscoring its strain-specific action. Kamal et al. echoed this in their study, where polyphenol-rich extracts had inconsistent effects on *L. casei*, suggesting potential bacterial defense mechanisms that may reduce herbal efficacy.[24]

CHX, on the other hand, caused extensive lysis and collapse of *L. casei* cell structures, indicating high effectiveness. These results align with Singh et al., who highlighted the strong bactericidal action of CHX on *L. casei*, though they also observed possible adaptive resistance in some strains.[25]

Biofilm Disruption Insights:
Biofilm resilience is a critical factor in dental caries progression. The present study demonstrated that MHPE significantly disrupted the biofilm matrix of *S. mutans*, as visualized under SEM.[26] This capacity to penetrate and destabilize the protective matrix suggests MHPE's bioactive compounds effectively interfere with biofilm architecture.[27] On the other hand, CHX displayed a comparatively limited impact on *S. mutans* biofilms, likely due to biofilm-mediated resistance that reduces agent penetration and efficacy.[4]

These findings advocate for MHPE as a natural alternative or adjunct to CHX, particularly in targeting *S. mutans*, a key contributor to plaque and caries formation.[1] The study not only reaffirms the antimicrobial efficacy of herbal compounds but also provides SEM-based evidence of their morphological effects, which is crucial for understanding their mechanisms of action.

In summary, the MBC analysis validates MHPE's bactericidal potential, especially against *S. mutans*, and places it within a comparable range to other effective herbal formulations cited in previous studies. Although CHX still shows superior bactericidal action in terms of lower MBC values, MHPE presents a compelling natural alternative with multi-targeted effects and fewer side effects. The present findings contribute to the growing evidence base supporting the integration of traditional herbal medicine in modern oral healthcare protocols.[11,12,13]

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.

REFERENCES

1. Ramalingam K, Amaechi BT. Antimicrobial effect of herbal extract of Acacia arabica with triphala on biofilm-forming cariogenic microorganisms. J Ayurveda Integr Med. 2020;11(3):322-8.
2. De Zoysa MHN, Rathnayake H, Hewawasam RP, Wijayaratne WMDGB. Determination of in vitro antimicrobial activity of five Sri Lankan medicinal plants against selected human pathogenic bacteria. Int J Microbiol. 2019; 2019:1-8.
3. Elgamily H, Safy R, Makharita R. Influence of medicinal plant extracts on growth of oral pathogens Streptococcus mutans and Lactobacillus acidophilus: An in-vitro study. Open Access Maced J Med Sci. 2019;7(14):2328-34.
4. Stepanovic S, Cirkovic I, Ranin L, Svabic-Vlahovic M. Biofilm formation by Salmonella spp. and Listeria monocytogenes on plastic surface. Lett Appl Microbiol. 2004;38(5):428-32.
5. Cavalcanti A, Da Silva N, Alexandria Adílis KF, De Lima A, Claudino L, De Oliveira Carneiro T, et al. In vitro antimicrobial activity of mouthwashes and herbal products against dental biofilm-forming bacteria. Contemp Clin Dent. 2012;3(3):302.
6. Turki O, Jafar Z. Antibacterial activity of Juglans regia L. dry husk extract against Streptococcus mutans and Lactobacillus: An in vitro study. Dent Hypotheses. 2023;14(1):29.
7. S. V, Mohana Murali S, Sivasamy S, Elizabeth Chaly P. Evaluation of antimicrobial efficacy of Andrographis paniculata (Nilavembu decoction) against various microbial biofilms formed on the tooth substrate - An in vitro study. J Clin Lab Med. 2023;11(1):580-93.
8. Hu S, Li S, Xu Y, Huang X, Mai Z, Chen Y, Xiao H, Ning W, Gaus S, Savkovic V, Lethaus B, Zimmerer R, Acharya A, Ziebolz D, Schmalz G, Huang S, Zhao J, Hu X. The antitumor effects of herbal medicine Triphala on oral cancer by inactivating PI3K/Akt signaling pathway: based on the network pharmacology, molecular docking, in vitro and in vivo experimental validation. Phytomedicine. 2024 Jun;128:155488.
9. Assis FFV, Silva NCD, Moraes WP, Barata LES, Minervino AHH. Chemical Composition and In Vitro Antiplasmodial Activity of the Ethanolic Extract of *Cyperus articulatus* var. *nodosus* Residue. Pathogens. 2020 Oct 27;9(11):889.
10. Bazaid AS, Alsolami A, Patel M, Khateb AM, Aldarhami A, Snoussi M, Almusheet SM, Qanash H. Antibiofilm, Antimicrobial, Anti-Quorum Sensing, and Antioxidant Activities of Saudi Sidr Honey: In Vitro and Molecular Docking Studies. Pharmaceutics. 2023 Aug 22;15(9):2177.
11. Vahabi S, Najafi E, Alizadeh S, Gharehaghaji Z. In vitro antimicrobial effects of some herbal essences against oral pathogens. *Afr J Microbiol Res*. 2021;5(18):2820-2824. doi:10.5897/AJMR11.588.
12. Kiryakova Y, Topalova Y, Dimkov R, Kirova G, Topalova D. The antimicrobial sensitivity of *Streptococcus mutans* and *Streptococcus sanguis* to nanoparticles andchlorhexidine.*JIMAB*.2015;21(1):705-710.
13. Ghorbani M, Salari S, Nourian A, Mohammadi R, Ghalehno M. Clinical evaluation of *Magnolia Grandiflora* bark mouthwash on *S. mutans* reduction. *Clin Oral Investig*. 2021;25(6):4123-4131.
14. Koo H, Hayacibara MF, Schobel BD, Cury JA, Rosalen PL, Park YK, et al. Inhibition of *Streptococcus mutans* biofilm accumulation and polysaccharide production by apigenin and tt-farnesol. *J Antimicrob Chemother*. 2003;52(5):782-9. doi:10.1093/jac/dkg449.
15. Kiryakova Y, Topalova Y, Dimkov R, Kirova G, Topalova D. The antimicrobial sensitivity of *Streptococcus mutans* and *Streptococcus sanguis* to nanoparticles andchlorhexidine. *JIMAB*.2015;21(1):705-710. doi:10.5272/jimab.2015211.705
16. Radmand H, Shariati M, Mohammadi F, Salehi A. Nanoliposomal formulation of *Punica granatum* extracts for improved antibiofilm efficacy against *S. mutans*. *BMC Oral Health*. 2024;24(1):53.
17. Vaishnavi K, Priya K, Balachandran A. Evaluation of *Andrographis paniculata* (Nilavembu decoction) against microbial biofilms on dental substrates. *J Herb Med*. 2023;42:125478.
18. Ghorbani M, Salari S, Nourian A, Mohammadi R, Ghalehno M. Clinical evaluation of *Magnolia Grandiflora* bark mouthwash on *S. mutans* reduction. *Clin Oral Investig*. 2021;25(6):4123-4131.
19. AlMatar M, AlMandeal H, Var I, AlMowallad A, AlYousef SA, AlAqeel T, et al. Antimicrobial efficacy of green-synthesized silver nanoparticles against dental cariogenic microorganisms. *Saudi Dent J*. 2022;34(1):13-20. doi: 10.1016/j.sdentj.2021.06.004.
20. Kiryakova Y, Topalova Y, Dimkov R, Kirova G, Topalova D. The antimicrobial sensitivity of *Streptococcus mutans* and *Streptococcus sanguis* to colloidal solutions of different nanoparticles applied as mouthwashes. *J IMAB*. 2015;21(1):705-710. doi:10.5272/jimab.2015211.705.
21. Jiayi L, Xu M, Zhang H, Wang Y. Chlorhexidine susceptibility and biofilm tolerance of Streptococcus mutans clinical isolates. *J Dent Res*. 2022;101(8):978-985. doi:10.1177/00220345221107984
22. Mensitieri F, Falasca F, di Carlo D, Montemurro F, Mattei A. Antibiofilm activity of commercial chlorhexidine mouthwashes against Streptococcus mutans: An SEM and microbiological analysis. *J Clin Med*. 2023;12(4):1302. doi:10.3390/jcm12041302
23. Gaurav A, Prasad S, Kumar N, Sharma P, Chauhan S. Antibacterial and antibiofilm potential of polyherbal extracts against Lactobacillus casei and Streptococcus mutans. *J Appl Microbiol*. 2021;131(2):785-795. doi:10.1111/jam.15044
24. Kamal N, Verma S, Mishra P, Patel R. Evaluation of herbal antimicrobials against Lactobacillus casei biofilms: A comparative SEM-based study. *Front Microbiol*. 2023; 14:1165284. doi:10.3389/fmicb.2023.1165284
25. Singh R, Patel M, Sharma N, Kapoor R. Chlorhexidine resistance in Lactobacillus casei biofilms: A morphological and genetic analysis. *Microb Pathog*. 2023; 180:106152. doi: 10.1016/j.micpath.2023.10615
26. Khalid M, Hassani D, Bilal M, Butt ZA, Hamayun M, Ahmad A. (2017).Antimicrobial activity of selected medicinal plants against dental biofilm forming bacteria. *International Journal of Phytomedicine*, **9**(3), 112-124.
27. Pathan MM, Bhat KG, Joshi VM. (2017). Comparative antimicrobial effectiveness of herbal and chlorhexidine mouthwash. *Journal of Periodontal Research*, **52**(4), 367-375.