Open Access

ORIGINAL ARTICLE

Comparative in-vitro evaluation of antimicrobial efficacy of herbal extracts versus chlorhexidine against oral Staphylococcus aureus isolates in smokers

Saad Shahid[1] , Muhammad Munir[1] , Mirza Ameer Faizan Ali[1]

Department of Microbiology, Azra Naheed Medical College, Superior University Lahore, Pakistan[1]

ABSTRACT

Background: Staphylococcus aureus is an opportunistic oral pathogen that can colonize in smokers due to alterations in the oral microbiome.

Objective: To assess and compare the antimicrobial effectiveness of herbal extracts (Azadirachta indica (neem), Syzygium aromaticum (clove oil), Ocimum tenuiflorum (Tulsi), and Camellia sinensis (green tea)) versus chlorhexidine against Staphylococcus aureus in smokers.

Methods: This in vitro experimental study was conducted at Azra Naheed Medical and Dental College from December 2025 to March 2026 on 100 adult smokers. Dental plaque samples were collected from 100 adult smokers, from which Staphylococcus aureus isolates were obtained and tested. Herbal extracts of Azadirachta indica (neem), Syzygium aromaticum (clove oil), Ocimum tenuiflorum (Tulsi), and Camellia sinensis (green tea) were prepared using ethanol extraction and compared for antimicrobial activity using the agar well diffusion method. Chlorhexidine (0.2%) was used as a control. Mean inhibition zones were compared with one-way-ANOVA.

Results: Chlorhexidine showed the largest inhibition zone (24.70±3.57), followed by clove oil (19.40±4.11), green tea (14.10±3.92), Tulsi (15.30±3.25), and Neem showed the smallest zone (12.01±2.58mm) ($p<0.001$). There was a significant difference in mean inhibition zones between chlorhexidine and clove ($p<0.001$), whereas no significant difference was observed between green tea and Tulsi ($p=0.116$). An inverse correlation was observed between the number of cigarettes smoked per day and the zone of inhibition for green tea ($r=-0.213$, $p=0.033$), but no significant correlation was observed with chlorhexidine ($r=0.029$, $p=0.778$).

Conclusion: Chlorhexidine demonstrated the greatest antimicrobial efficacy against oral S. aureus isolates from smokers, followed by clove oil, green tea, Tulsi, and neem. Further optimization of herbal agent concentrations may enhance their clinical applicability.

Key Words: Antimicrobial efficacy, Chlorhexidine, Medicine, Herbal, Smoking, Staphylococcus aureus

DOI: https://doi.org/10.53685/jshmdc.v7i1.454

Corresponding Author:
Saad Shahid
Post Graduate Resident
Department of Microbiology, Azra Naheed Medical College, Superior University Lahore, Pakistan
Email address: drcsaadshahid@gmail.com
Received: 11. 4.2026, 1st Revision:18. 05.2026
2nd Revision: 25.05.2026, Accepted: 30.05.26

How to cite this article: Shahid S, Munir M, Ali MAF. Comparative in-vitro evaluation of antimicrobial efficacy of herbal extracts versus chlorhexidine against oral Staphylococcus aureus isolates in smokers. J Shalamar Med Dent Coll. 2026; 7(1): 06-12. doi: https://doi.org/10.53685/jshmdc.v7i1.454

INTRODUCTION

Antibacterial resistance (AMR) is a global challenge, with the World Health Organization (WHO) designating it a top priority. AMR compromises the effectiveness of treatment for various infections, especially hospital-based infections, making even routine procedures riskier.[1] *Staphylococcus* aureus (S. aureus) is a major contributor to methicillin-resistant S. aureus, a major cause of hospital and community-acquired infections, which frequently colonizes the nasal and oral cavity, especially in immunocompromised patients, increasing the risk of transmission and systemic infections.[2]

Smoking is a risk factor for oral diseases, which reportedly affect the oral microbiome, increasing S.

aureus colonization, as tobacco smoke can create a favorable environment for bacterial growth by lowering oxygen levels, decreasing salivary flow, and altering pH, thereby promoting the growth and resistance of oral bacteria.[3]

Another reason for antimicrobial resistance, especially in clinical settings, is the overuse and misuse of antibiotics, prompting the search for alternative medicines to combat oral pathogenic bacteria.[4] Herbal products have a long history of use in traditional medicine, offering a potential solution to overuse and misuse of antibiotics and alternative medicines.[5] Azadirachta indica, also known as neem; Syzygium aromaticum, also called clove oil; Ocimum tenuiflorum, commonly called Tulsi; and Camellia sinensis, also called green tea, have been reported to have antibacterial activity in the literature.[6] These plant-based agents are rich in bioactive compounds such as eugenol in clove oil, polyphenols in green tea, and nimbolide in neem and reportedly exert antibacterial activity through membrane disruption, inhibition of protein synthesis, and interference with biofilm formation.[7, 8]

In Pakistan, smoking has a high prevalence, warranting an urgent need for effective oral hygiene using herbal products to serve as antimicrobial agents against pathogens like S. aureus, especially where resources are limited.[2] Previous in-vitro studies and reviews have reported antimicrobial activity of neem, clove oil, Tulsi, and green tea against oral pathogens and *S. aureus*.[3, 9-11]

Previous studies have evaluated these agents individually, against standard laboratory strains, or in non-smoker populations. Evidence remains limited on the comparative efficacy of these herbal extracts against oral S. aureus isolates from smokers, despite smoking-related alterations in the oral microbiome that may influence bacterial colonization and antimicrobial susceptibility.[9,11] Therefore, the present study provides smoker-specific comparative data by evaluating neem, clove oil, Tulsi, and green tea against oral S. aureus isolates, with chlorhexidine as the control. Despite the wide use of herbal products in traditional medicine, there is limited data regarding their effectiveness, especially in smokers.[3]

The study aimed to assess and compare the antimicrobial effectiveness of herbal extracts (Azadirachta indica (neem), Syzygium aromaticum (clove oil), Ocimum tenuiflorum (Tulsi), and Camellia sinensis (green tea) versus chlorhexidine against S. aureus in smokers.

METHODS

This in vitro experimental study was conducted from December 2025 to March 2026, following approval from the Institutional Ethics Board. The experimental work was carried out in the Pathology department of Azra Naheed Medical College, Superior University, Lahore, Pakistan. Sample size was calculated using a two-sided alpha level of 0.05 and 80% power; the minimum number of isolates required to detect an expected mean difference of 5.0 mm in the zone of inhibition between agents (based on previously reported agar diffusion data, with SD=6.93 mm) was 15 isolates. To improve the precision and reliability of findings, the sample size was increased to 100 isolates, with each isolate evaluated against all agents. A non-probability consecutive sampling technique was used. Eligible adult smokers who fulfilled the selection criteria and provided written informed consent were recruited until the required sample size of 100 participants was achieved. The inclusion criteria were adult smokers aged 18 years and above, with a history of cigarette smoking for at least one year, presence of dental plaque, and provided written informed consent. Participants were included only if oral plaque samples yielded confirmed S. aureus isolates. Participants who had used systemic antibiotics, antimicrobial mouthwash, or professional periodontal therapy within the preceding four weeks were excluded. Individuals with systemic conditions affecting oral immunity, active oral infections requiring immediate treatment, or incomplete clinical/smoking history were also excluded.

Written informed consent, followed by a self-administered questionnaire targeting demographics and smoking habits, was obtained from all participants before sample collection. The dental plaque was collected from four teeth, i.e., the upper right first molar, the upper left central incisor, the lower left first molar, and the lower right central incisor. The plaque was pooled in a single sterile tube containing sterile saline and transported for testing within 4 hours of collection. The samples were vortexed for 30s, and serial dilutions were prepared in phosphate-buffered saline (PBS). Aliquots of 100 μL from the appropriate dilutions were plated onto blood Agar and Mannitol Salt Agar (MSA) and incubated at 37°C for 24 to 48 hours. The S. aureus appeared golden yellow on Blood Agar and yellow on MSA.

Gram staining was done, where S. aureus appeared as Gram-positive cocci in irregular grape-like clusters. The catalase test was done, showing bubbles when hydrogen peroxide was added to the bacterial culture, indicating a positive catalase reaction. The coagulase test was done using rabbit plasma, and clot formation indicated the presence of S. aureus.

The herbal products i.e., Neem, Clove oil, Tulsi, and Green tea were purchased from authenticated suppliers and the bioactive compounds were extracted using ethanol solvent extraction where herbal agents were dried and ground and a 1:10 (w/v) ratio of dried powder to 70% ethanol were mixed, and shaked at room temperature for 48 to 72 hours, and later concentrated using a rotary evaporator below 50°C, and dried at 40°C, to form solid extracts, which were reconstituted in sterile distilled water to prepare stock solutions at concentrations of 100 mg/ml.

Minimum inhibitory concentration (MIC) testing was performed using tube dilution to guide selection of herbal extract concentrations for agar well diffusion testing. One confirmed oral S. aureus isolate was selected for baseline MIC. Serial dilutions of ethanolic extracts of neem, Tulsi, green tea, and clove oil were prepared in Mueller-Hinton broth. A bacterial inoculum was prepared from a fresh overnight culture and adjusted to 0.5 McFarland standard. The standardized inoculum was added to each tube. The tubes were incubated at 37°C for 24 hours. MIC was defined as the lowest concentration of the extract that showed no visible turbidity compared with the positive control. The MIC values were 25% for neem, 5% for Tulsi, 5% for green tea, and 4% for clove oil; therefore, these MIC-guided concentrations were selected for subsequent agar well diffusion testing.

The antimicrobial activity of the herbal extracts was assessed using the agar well diffusion method. Fresh overnight cultures of confirmed oral S. aureus isolates were prepared, and bacterial suspensions were adjusted to match the 0.5 McFarland standard. Mueller-Hinton Agar plates were inoculated evenly using sterile cotton swabs. 6 mm-diameter wells were punched into the inoculated agar using a sterile cork borer, and 100 µL of each herbal extract was added to the corresponding wells at MIC-guided concentrations: neem 25%, Tulsi 5%, green tea 5%, and clove oil 4%. For clove oil, 1% v/v Tween-80 was added to support emulsification and diffusion in the agar medium. Chlorhexidine 0.2% was used as the control. The plates were incubated at 37°C for 24 hours, and zones of inhibition around each well were measured in millimeters using a transparent ruler.

Ethical Approval

The study was approved by the Ethical Review Board of Azra Naheed Medical College on 1st December, 2025 (Ref no: FRB/BMS/02).

Statistical Analysis

The data were analyzed using SPSS version 26, and the descriptive statistics were calculated for each variable. The quantitative variables were age, smoking duration, cigarettes smoked per day, and zone of inhibition for each agent. The normality was assessed using the Shapiro–Wilk test; the data were normally distributed. The differences in inhibition zones were assessed using one-way ANOVA, followed by Tukey's HSD post hoc test for pairwise comparisons. Pearson correlation was applied for smoking and zone of inhibition correlation. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

A total of 100 smokers were included in the study, with a mean age of 36.62±12.88 years. The average smoking duration was 8.17±7.63 years, and participants smoked an average of 16.60±4.91 cigarettes per day. The antimicrobial activity of the herbs against S. aureus was evaluated by measuring the zones of inhibition.

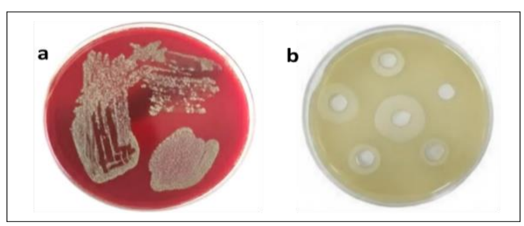

Figure 1: *(a) Isolated Staphylococcus aureus. (b) Zone of inhibition for Staphylococcus aureus*

Chlorhexidine showed the largest zone of inhibition, followed by Clove oil. The inhibition zones of Green tea and Tulsi were moderate, whereas Neem showed the smallest (Table 1).

The neem inhibition zones were significantly smaller than those of all other agents (p < 0.001). There was a significant difference in mean inhibition zones between chlorhexidine and clove (p<0.001), whereas no significant difference (p=0.116) was observed between green tea and Tulsi inhibition zones (Table 2).

Table 1: Comparison of mean zones of inhibition

Agents	n	Mean ± SD	p value
Chlorhexidine	100	24.70±3.57	
Clove Oil	100	19.40±4.11	
Tulsi	100	15.30±3.25	<0.001*
Green Tea	100	14.10±3.92	
Neem	100	12.01±2.58	

*One-way ANOVA test was applied. *p<0.05 was taken as statistically significant*

Table 2: Pairwise comparisons of mean inhibition zones among tested agents

Comparison	mean difference (mm)	p value
Clove Oil -Tulsi	4.10	<0.001*
Clove Oil - Green Tea	-5.30	<0.001*
Clove Oil - Neem	-7.38	<0.001*
Clove Oil - Chlorhexidine	5.30	<0.001*
Chlorhexidine - Green Tea	-10.60	<0.001*
Chlorhexidine - Neem	12.68	<0.001*
Chlorhexidine - Tulsi	-9.40	<0.001*
Tulsi - Green Tea	1.20	0.116
Tulsi -. Neem	3.28	<0.001*
Neem - Green Tea	-2.08	<0.001*

*Tukey's post hoc test applied. * p<0.05 was taken as statistically significant.*

Table 3: Correlation of smoking variables with zones of inhibition among tested agents

Smoking variable	Agent	*r value*	*p value*
Cigarettes smoked per day	Chlorhexidine	0.029	0.778
	Clove oil	−0.034	0.734
	Tulsi	−0.052	0.608
	Green tea	−0.213	0.033*
	Neem	−0.072	0.478
Smoking duration	Chlorhexidine	−0.027	0.792
	Clove oil	−0.017	0.868
	Tulsi	−0.001	0.991
	Green tea	−0.054	0.594
	Neem	−0.186	0.064

*Pearson correlation test applied. *p<0.05 was considered statistically significant.*

There was a significant negative correlation between the number of cigarettes smoked per day and the green tea zone of inhibition (p=0.033). No significant correlation was observed between cigarettes smoked per day and the zones of inhibition for chlorhexidine, clove oil, Tulsi, or neem. Smoking duration was not significantly associated with the zones of inhibition for any of the tested agents (Table 3).

Based on the antimicrobial testing, the inhibitory activity was highest for chlorhexidine, followed by clove oil, Tulsi, green tea, and neem. Clove oil was the most effective herbal agent, while Tulsi and green tea showed moderate antibacterial activity and were not statistically different from each other. Neem showed the lowest inhibitory activity among the tested agents.

DISCUSSION

This in-vitro experimental study was conducted on adult smokers. Dental plaque samples were collected from smokers, from which Staphylococcus aureus isolates were obtained and tested. Herbal extracts of Azadirachta indica (neem), Syzygium aromaticum (clove oil), Ocimum tenuiflorum (Tulsi), and Camellia sinensis (green tea) were compared for antimicrobial activity using the agar well diffusion method. Chlorhexidine (0.2%) was used as a control.

Chlorhexidine showed the highest inhibitory activity against oral *Staphylococcus* aureus isolates from smokers, followed by clove oil. Tulsi and green tea that showed moderate inhibitory activity and were not statistically different from each other, while neem showed the lowest activity among the tested agents. A higher number of cigarettes smoked per day was weakly associated with reduced inhibitory activity of green tea, whereas no significant association was observed for the other agents. These findings aligned with prior literature due to the superior antibacterial properties.[13,14] Chlorhexidine is a cationic bisbiguanide that binds electrostatically to negatively charged bacterial cell walls and oral surfaces, giving it prolonged retention in the oral cavity. Chlorhexidine has broad-spectrum activity due to its ability to disrupt bacterial cell membranes and inhibit protein synthesis.[13] Prolonged use of chlorhexidine can lead to tooth staining, altered taste, and increased risk of antibiotic resistance, underscoring the need for alternative natural antimicrobials with fewer side effects and oral benefits.[15] Clove oil showed a significantly smaller inhibition zone compared to chlorhexidine but was one of the most effective herbs, and this finding aligns with recent studies reporting that clove oil, due to eugenol as an active ingredient, has strong antimicrobial activity against *S.*

aureus by membrane disruption, interference with protein synthesis in bacteria, and the inhibition of biofilm.[16,17] Clove oil reportedly has better antimicrobial activity than other herbs like neem or Tulsi.[18] Its slightly lower efficacy than chlorhexidine may be attributed to the concentration and solubility of eugenol in the oral product preparation, as well as the slower release of active compounds in aqueous solutions. Despite this, the relatively low risk of side effects associated with clove oil makes it a promising natural alternative to synthetic antimicrobial agents.[19]

Tulsi and green tea showed a moderate zone of inhibition, consistent with the literature reporting the antibacterial properties of green tea due to polyphenolic compounds such as epigallocatechin gallate, which can disrupt bacterial membranes, inhibit enzymes, and hinder biofilm formation.[20] Previous studies have reported green tea activity against *S. aureus* in vitro, with some studies also reporting a synergistic effect when used in conjunction with conventional antibiotics.[21, 22]

The current study reported a negative correlation between the number of cigarettes and the efficacy of green tea, indicating that increased smoking may reduce the effectiveness of green tea against *S. aureus*. This could be due to the effects of smoking on the oral microbiome, making bacteria such as *S. aureus* less susceptible and more resistant.[21] This finding supports the notion that smoking creates a more adverse environment for antimicrobial agents and may diminish their effectiveness.[23] However, this finding should be interpreted cautiously because the study did not include a non-smoker comparator group and does not establish that smoking directly reduces the antimicrobial effectiveness of green tea. Like green tea, Tulsi contains eugenol and carvacrol, which have antibacterial properties.[24] Recent data suggest that Tulsi extracts can inhibit bacterial growth by damaging the cell membrane and modulating the host immune response.[25] However, the efficacy of Tulsi and green tea was not as strong as that of chlorhexidine or clove oil. The findings suggest that Tulsi and green tea may be used as an adjunct or as a combination therapy rather than as a single agent.[9]

Neem (Azadirachta indica) exhibited the lowest antimicrobial activity, consistent with previous studies reporting variable antibacterial effects of neem that depend on the extraction method and the concentration of the herb used.[11] Neem has been used for its antimicrobial properties, but its efficacy is weaker than that of other natural agents like clove oil or chlorhexidine. The low effectiveness of neem in this study could be due to the extraction method, the extract concentration, and variation in bioactive components; therefore, neem can be used as an adjunctive for plaque control and gingivitis, though further optimization is required to become an effective standalone agent.[8, 11]

The study reported that the duration of smoking did not affect the herbs' antimicrobial efficacy, which aligns with the literature indicating that the effects of smoking on oral bacteria depend on factors such as smoking frequency and intensity and oral hygiene practices.[23,24] However, the number of cigarettes per day in this study reduced the effectiveness of green tea against *S. aureus*. This finding is consistent with a previous study indicating that smoking can alter the oral microbiome, creating conditions leading to antibiotic resistance.[25]

CONCLUSION

Chlorhexidine demonstrated the greatest antimicrobial efficacy against oral *S. aureus* isolates from smokers, followed by clove oil, green tea, Tulsi, and neem. Smoking reduced the effectiveness of green tea, whereas no significant effect was observed for the other agents. These findings suggest that certain herbal products have antimicrobial potential and may serve as adjuncts or potential alternatives to synthetic agents; however, their effectiveness in smokers varies. Further optimization of herbal agent concentrations and clinical evaluation are needed to enhance their applicability.

Limitations and future recommendations

This in vitro study used the agar well diffusion method, which may not fully replicate oral conditions, including saliva, pH variations, and dental plaque biofilms. Inhibition zones may also be influenced by the diffusion properties of the tested agents. Only the zone of inhibition was assessed, while minimum bactericidal concentrations (MBCs) and biofilm activity were not evaluated, and the study was limited to smokers. Future studies should include in vivo and clinical investigations, assess MBCs and biofilm activity, and evaluate different concentrations and combinations of herbal extracts.

REFERENCES

1. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022; 399(10325): 629-655. doi: 10.1016/S0140-6736(21)02724-0
2. Razzaq S, Nagi MLF, Athar U, Kazmi T, Alslamah T, Naz S, et al. Prevalence of tobacco consumption and the associated factors among the adults in an urban slum: findings from the WHO STEPwise survey. Tob Induc Dis. 2022; 20: 91. doi: 10.18332/tid/154636
3. Cátala-Valentín AR, Almeda J, Bernard JN, Cole AM, Cole AL, Moore SD, et al. E-cigarette aerosols promote oral S. aureus colonization by delaying an immune response and bacterial clearing. Cells. 2022; 11(5): 773. doi:10.3390/cells11050773
4. Buonavoglia A, Leone P, Solimando AG, Fasano R, Malerba E, Prete M, et al. Antibiotics or no antibiotics, that is the question: an update on efficient and effective use of antibiotics in dental practice. Antibiotics. 2021; 10(5): 550. doi: 10.3390/antibiotics10050550
5. Tan S, Guo H, Chow A. Complementary and alternative medicine (CAM) use and its association with antibiotic usage practices: A nationally representative population study. Int J Antimicrob Agents. 2023; 61(6): 106796. doi: 10.1016/j.ijantimicag.2023.106796
6. Subramanian S, Shenoy PA, Pai V. Antimicrobial activity of some Essential oils and Extracts from Natural sources on Skin and Soft tissue infection causing microbes: An In-vitro Study. Res J Pharm Technol. 2021; 14(7): 3603-3609. doi: 10.52711/0974-360X.2021.00623
7. Touati A, Mairi A, Ibrahim NA, Idres T. Essential Oils for Biofilm Control: Mechanisms, Synergies, and Translational Challenges in the Era of Antimicrobial Resistance. Antibiotics. 2025; 14(5): 503. doi: 10.3390/antibiotics 14050503
8. Arzani V, Soleimani M, Fritsch T, Jacob UM, Calabrese V, Arzani A. Plant polyphenols, terpenes, and terpenoids in oral health. Open Med (Wars). 2025; 20(1): 20251183. doi: 10.1515/med-2025-1183
9. Deepika B, Ramamurthy J, Jayakumar ND, Kumar SR. Comparative clinical data for gingivitis treatment using gels from Ocimum sanctum (Tulsi) and chlorhexidine (CHX). Bioinformation. 2021; 17(12): 1091-1098. doi: 10.6026/973206300171091
10. Nagappan N, Palaneeswaran K, Nalankilli AV, Nyklesh V, Mani M, Aziz MBA. Antibacterial efficacy of herbal and 0.2% chlorhexidine mouth rinse against oral pathogens–An in vitro study. J Pharm Bioallied Sci. 2024; 16(Suppl 2): S1453-S1455. doi: 10.4103/jpbs.jpbs_858_23
11. Wylie MR, Merrell DS. The antimicrobial potential of the neem tree Azadirachta indica. Front Pharmacol. 2022; 13: 891535. doi: 10.3389/fphar.2022.891535
12. Shakhatreh MAK, Khabour OF, Alzoubi KH, Masadeh MM, Hussein EI, Bshara GN. Alterations in oral microbial flora induced by waterpipe tobacco smoking. Int J Gen Med. 2018: 11: 47-54. doi: 10.2147/IJGM.S150553
13. Bartsch S, Kohnert E, Kreutz C, Woelber JP, Anderson A, Burkhardt AS, et al. Chlorhexidine digluconate mouthwash alters the oral microbial composition and affects the prevalence of antimicrobial resistance genes. Front Microbiol. 2024; 15: 1429692. doi: 10.3389/fmicb.2024.1429692
14. Chavarría-Bolaños D, Esparza-Villalpando V, Ramírez K. Antibacterial and antifungal capacity of three commercially available mouthwashes with different concentrations of chlorhexidine. Odovtos Int J Dent Sci. 2022; 24(2): 69-80. doi: 10.15517/IJDS.2021.48079
15. Kamdem GSJN, Toukam M, Ntep DBN, Kwedi KGG, Brian NZ, Fokam ST, et al. Comparison of the effect of saline mouthwash versus chlorhexidine on oral flora. Adv Oral Maxillofac Surg. 2022; 6: 100273. doi: 10.1016/j.adoms.2022.100273
16. Lee J-S, Choi YS, Lee HG. Synergistic antimicrobial properties of nanoencapsulated clove oil and thymol against oral bacteria. Food Sci Biotechnol. 2020; 29(11): 1597-1604. doi: 10.1007/s10068-020-00803-w
17. Li J, Li C, Shi C, Aliakbarlu J, Cui H, Lin L. Antibacterial mechanisms of clove essential oil against Staphylococcus aureus and its application in pork. Int J Food Microbiol. 2022; 380: 109864. doi: 10.1016/j.ijfoodmicro
18. Fatima S, Saba S, Hussain A, Aslam L, Naveed A. Antibacterial and anti-biofilm profiling of Syzygium aromaticum plant extracts against multi-drug resistant human pathogens. Pak J Biochem Biotechnol. 2023; 4(2): 71-82. doi: 10.52700/pjbb.v4i2.236
19. Hiwandika N, Sudrajat SE, Rahayu I. Antibacterial and antifungal activity of clove extract (Syzygium aromaticum). Eureka Herba Indones. 2021; 2(2): 86-94. doi: 10.37275/ehi.v2i2.18
20. Hussain M, Riasat M, Nisa WU, Khattak I, Ullah F, Shaid F. Assessment of effect of cigarette smoking on periodontal health of patients reporting to dental section of Bacha Khan Medical College Mardan. Pak J Public Health. 2020; 10(3): 185-189. doi: 10.32413/pjph.v10i3.506
21. Kong C, Zhang H, Li L, Liu Z. Effects of green tea extract epigallocatechin-3-gallate (EGCG) on oral disease-associated microbes: A review. J Oral Microbiol. 2022; 14(1): 2131117. doi: 10.1080/20002297.2022.2131117
22. Sartini S, Djide MN, Amir MN, Permana AD. Phenolic-rich green tea extract increases the antibacterial activity of amoxicillin against Staphylococcus aureus by in vitro and ex vivo studies. J Pharm Pharmacogn Res. 2020; 8(6): 491-500. doi: 10.56499/jppres20.844_8.6.491
23. Stapleton EM, Manges R, Parker G, Stone EA, Peters TM, Blount RJ, et al. Indoor particulate matter from smoker homes induces bacterial growth, biofilm formation, and impairs airway antimicrobial activity. A pilot study. Front Public Health. 2020; 7: 418. doi: 10.3389/fpubh.2019.00418
24. Kowalewska A, Majewska-Smolarek K. Eugenol-based polymeric materials antibacterial activity and applications. Antibiotics. 2023; 12(11): 1570. doi: 10.3390/antibiotics12111570
25. Rahman RTA, Kamal N, Mediani A, Farag MA. How do herbal cigarettes compare to tobacco? A comprehensive review of their sensory characters, phytochemicals, and functional properties. ACS Omega. 2022; 7(50): 45797-45809. doi: 10.1021/acsomega.2c04708

AUTHORS' CONTRIBUTION:

SS: Conception of the study, data acquisition, analysis, manuscript drafting, final approval
MM: Conception of the study, data collection, analysis & interpretation, critical review
MAFA: Data collection, data analysis, manuscript drafting, and final review

All Authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

CONFLICT OF INTEREST:
All authors declared no conflict of interest.

GRANT SUPPORT AND FINANCIAL DISCLOSURE:
No specific grant was taken for this research from any funding agency in the public, commercial or not-for-profit sectors.

DATA SHARING STATEMENT:
The data are available from the corresponding author upon reasonable request.
……………………………………………………………………………………
……………………………………………………………………………………