ORIGINAL RESEARCH

Clinical Assessment of *Syzygium cumini* Seed Extract as a Natural Antimicrobial Agent for Modulating Bacterial Aerosol Dissemination during Ultrasonic Scaling: A Randomized Controlled Trial

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Abstract

Aim: The aim of this study was to assess the effects of *Syzygium cumini* seed extract mouthrinse on bacterial recovery from scaling-generated aerosols and compare it with chlorhexidine and saline.

Materials and methods: A randomized controlled clinical trial was conducted among 45 patients diagnosed with chronic gingivitis. Participants were assigned equally into three groups (group I, group II, and group III) ($n = 15$). In group I, participants rinsed with saline before ultrasonic scaling; in group II, participants rinsed with 0.2% chlorhexidine; and in group III, participants rinsed with *S. cumini* seed extract mouthrinse. Aerosolized microorganisms were collected on blood agar plates placed over the chest area, and colony-forming units (CFUs) were counted after incubation, that is, after 24 hours, and analyzed using analysis of variance (ANOVA) and the Games-Howell *post hoc* test.

Results: All 45 participants completed the study without adverse reactions. The *S. cumini* group demonstrated the lowest mean log CFU (2.44), followed by chlorhexidine (2.67) and saline (3.15). Statistical analysis showed a significant difference among the three groups ($p < 0.001$). The extract rinse showed a greater reduction in bacterial recovery from aerosols compared with saline, indicating a reduction in microbial load during ultrasonic scaling.

Conclusion: The normal saline group (group A) had the highest mean CFU value (3.15), followed by the chlorhexidine group (group B) with lower microbial counts (2.67). The lowest mean CFU value was recorded in the *S. cumini* seed extract group (group C) (2.44), indicating reduced microbial recovery following ultrasonic scaling.

Clinical significance: Ultrasonic scaling is considered one of the important aerosol-generating procedures due to the interaction between ultrasonic vibration and irrigation water. This increases the need for a natural and biocompatible alternative to reduce the microbial load. The current study proves that the use of *S. cumini* seed extract mouthrinse may provide a natural approach for reducing microbial aerosols during ultrasonic scaling procedures and may support infection control practices in dental settings.

Keywords: Microbial aerosol, Natural antimicrobial, *Syzygium cumini*, Ultrasonic scaling.

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Introduction

The COVID-19 pandemic has reinforced the importance of effective infection prevention strategies in dental practices, particularly due to the potential role of aerosols and splatter generated during clinical procedures in microbial dissemination.[1] Ultrasonic scaling is considered one of the important aerosol-generating procedures, as the interaction between ultrasonic vibration and irrigation water may facilitate the dispersion of microorganisms originating from saliva, dental biofilm, and the oral cavity into the surrounding environment.[2] These airborne microbial particles may contribute to environmental contamination and increase the possibility of cross-infection between dental health care workers and patients.[3]

Dental aerosols contain a heterogeneous microbial population, including cariogenic and periodontopathogenic organisms such as *Streptococcus mutans*, *Staphylococcus aureus*, and anaerobic species like *Porphyromonas gingivalis*. Since these microorganisms primarily originate from the patient's oral microbiota, reducing the available microbial load before aerosol-generating procedures may represent an additional preventive approach for improving clinical safety.[2]

Preprocedural oral rinsing has been suggested as a simple chairside method to temporarily decrease the number of

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microorganisms released during dental procedures. Chlorhexidine gluconate remains a frequently used antimicrobial rinse because of its broad-spectrum antimicrobial activity and substantivity. It exerts an antibacterial effect mainly by altering bacterial cell membranes and integrity, resulting in leakage of intracellular components and microbial death.[4] However, its repeated or prolonged use has been associated with adverse effects, including tooth staining, dysgeusia, mucosal irritation, and possible concerns regarding microbial adaptation.[4,5] These limitations have encouraged the investigation of naturally derived agents with antimicrobial activity and improved patient acceptability.

Among plant-derived therapeutic agents, *Syzygium cumini* (*Jamun*) has attracted attention due to its diverse phytochemical profile comprising tannins, flavonoids, gallic acid, and anthocyanins. These bioactive constituents have demonstrated antioxidant, antimicrobial, anti-inflammatory, and immunomodulatory properties, supporting the potential use of *S. cumini* in preventive oral healthcare applications.[6–8] Previous experimental and clinical studies have reported inhibitory effects of *S. cumini* extract against oral microorganisms, particularly cariogenic bacteria.[8,9]

Although various chemical and herbal mouthrinses have been investigated for their ability to reduce microbial dissemination during aerosol-generating dental procedures, the clinical applications of *S. cumini* seed extract specifically for controlling aerosolized bacterial load remain insufficiently explored.[9–13] Further evaluation is required to determine whether this herbal extract can provide effective microbial reduction comparable to conventional antimicrobial agents.

Therefore, this randomized controlled trial was designed to evaluate the effectiveness of *S. cumini* seed extract as a preprocedural mouthrinse in reducing bacterial aerosols generated during ultrasonic scaling and to compare its antimicrobial effect with 0.2% chlorhexidine and normal saline.

Materials and Methods

Study Design and Ethical Consideration

A randomized controlled clinical trial was conducted at Vivekanandha Dental College for Women, Tamil Nadu, India, from 2019 to 2020 to evaluate the effect of *S. cumini* seed extract as a preprocedural oral rinse on the microbial burden associated with ultrasonic scaling procedures. Ethical approval was obtained from the Institutional Ethics Committee (IEC) (IEC No. VDCW/IEC/167/2019), and the study was conducted under the Indian Council of Medical Research–Short-term Studentship (ICMR-STS) Programme (Ref. No. 2019-09133). The research methodology followed the ethical guidelines outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment.

Participant Selection and Sample Size Estimation

The required sample size was estimated based on previous clinical investigations assessing microbial changes following the use of preprocedural antimicrobial rinses. Considering 80% statistical power and a 95% confidence level, a minimum of 15 participants per group was determined to be adequate. The study included 45 participants diagnosed with chronic gingivitis within the age range of 18–35 years. A total of 45 eligible individuals were enrolled and randomly allocated into three groups (n = 15/group). Participants included in the study had at least 20 permanent teeth with adequate tooth distribution, plaque index scores ranging from 1.5 to 3, and periodontal probing sulcus depth not exceeding 3 mm. Participants were excluded if they presented with oral mucosal lesions, prosthetic appliances, systemic illness, recent antimicrobial therapy, or a history of hypersensitivity to chlorhexidine or plant-derived mouthrinse components.

This randomized controlled trial was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Fig. 1).

Preparation of *Syzygium cumini* Seed Extract

Mature seeds of *S. cumini* (*Jamun*), approximately 200 gm, were collected, taxonomically verified by a botanist, dried, and processed into a fine powder. A total of 10 gm of powdered seed material was dissolved in 100 mL of ethanol. The mixture was maintained at 40°C for 30 minutes with continuous agitation to enhance the extraction of bioactive constituents. Following extraction, the solution was centrifuged at 6000 rpm for 5 minutes, and the clarified extract was separated. The collected supernatant was concentrated by oven drying at 80°C. The dried extract was subsequently reconstituted to obtain the formulation used for clinical administration.

Randomization and Intervention

Participants were distributed equally among the three study groups using a simple randomization method by coin toss. A single-blinded trial was followed, in which the microbiological examiner involved in colony assessment was unaware of group allocation to reduce assessment bias. The assigned interventions were:

Group I: Participants rinsed with 10 mL of normal saline for 1 minute before scaling.

Group II: Participants rinsed with 10 mL of 0.2% chlorhexidine mouthrinse (Hexidine®, ICPA Health Products Ltd, Mumbai, India) for 1 minute.

Group III: Participants rinsed with 10 mL of *S. cumini* seed extract mouthrinse for 1 minute.

Aerosol Collection and Microbiological Assessment

Prior to each clinical procedure, the treatment area was prepared according to standard infection control measures to minimize environmental microbial interference. The participants were asked to rinse with the assigned solution in their respective group, that is, group I with 10 mL of normal saline, group II with 10 mL of 0.2% chlorhexidine mouthrinse, and group III with *S. cumini* seed extract. During ultrasonic scaling, sterile blood agar plates (HiMedia Laboratories Pvt Ltd, Mumbai, India) were positioned over the anterior chest region of each participant with adhesive tape to collect microorganisms dispersed through procedure-generated aerosols. Ultrasonic scaling was completed for all three groups by a single trained operator using a standardized ultrasonic scaler under consistent clinical conditions. Following completion of instrumentation, agar plates were immediately transferred for

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Conflict of interest: None

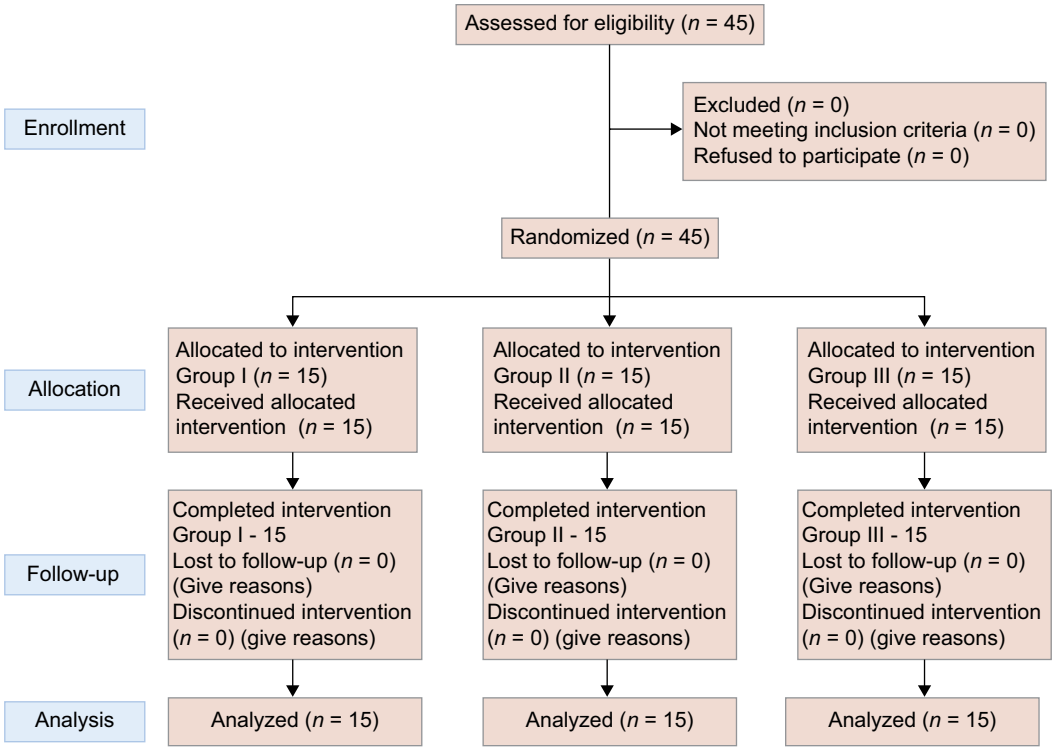

Fig. 1: CONSORT flow diagram

Table 1: Distribution and comparative analysis of log-transformed microbial recovery study groups

Group	*Mean*	*Min*	*Max*	*Shapiro–Wilk p*	*Levene's p*	*ANOVA p*	η^2
Group I (normal saline)	3.15	2.98	3.30	0.812	0.421	<0.001	0.412
Group II (chlorhexidine)	2.67	2.50	2.85	0.603			
Group III (*jamun* seed extract)	2.44	1.95	2.70	0.335			

*Statistically significant ($p < 0.05$). A large effect size ($\eta^2 = 0.412$) confirms strong practical significance

microbiological processing. The samples were incubated aerobically at 37°C for 24 hours. After incubation, visible colony-forming units (CFUs) were quantified by a blinded microbiologist. The recorded CFU values represented the microbial aerosol burden associated with each intervention group.

Outcome Measure

The primary outcome measure was the alteration in microbial aerosol load following administration of different preprocedural rinses. The aerosol-associated bacterial burden was assessed by comparing CFU counts recovered from blood agar plates after ultrasonic scaling.

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 25.0 (IBM Corp., Armonk, NY, USA). Because microbial colony counts demonstrate variability, CFU values were logarithmically transformed before analysis to improve data distribution and statistical reliability.

Descriptive statistics, including mean, minimum, and maximum values, were calculated for each group. Normal distribution of log-transformed CFU values was assessed using the Shapiro–Wilk test, while equality of variances was evaluated using Levene's test. Since the assumptions for parametric analysis were satisfied ($p > 0.05$), differences among the three groups were analyzed using one-way analysis of variance (ANOVA).

The magnitude of the relationship between the intervention and the reduction in bacterial count was assessed using eta squared (η^2) to estimate effect size. Differences between individual groups were further analyzed using the Games-Howell *post hoc* test. Statistical significance was interpreted at a p-value < 0.05, whereas $p < 0.01$ was regarded as highly significant.

Results

Participant Characteristics

All 45 participants completed the study and were included in the final analysis. No adverse reactions or complications associated with the study interventions were reported during the trial period.

Differences in log-transformed CFU counts were observed among the three intervention groups (Table 1). The normal saline group demonstrated the highest mean CFU value (3.15), whereas the chlorhexidine group exhibited lower microbial counts (2.67). The lowest mean CFU value was recorded in the *S. cumini* seed extract group (2.44), indicating reduced microbial recovery following ultrasonic scaling.

Evaluation of Data Distribution

Assessment of data distribution using the Shapiro–Wilk test showed p-values > 0.05 for all study groups, indicating that the transformed CFU values approximated a normal distribution. In addition,

Table 2: Pairwise comparison of microbial load between intervention groups

(I) Group	*(J) Group*	*Mean difference (I–J)*	*95% CI lower*	*95% CI upper*	*p-value*
Saline	Chlorhexidine	0.48021*	0.3650	0.5954	<0.001
Saline	*Jamun* extract	0.71234*	0.4892	0.9355	<0.001
Chlorhexidine	*Jamun* extract	0.23213*	0.0519	0.4124	0.015

*Statistically significant ($p < 0.05$)

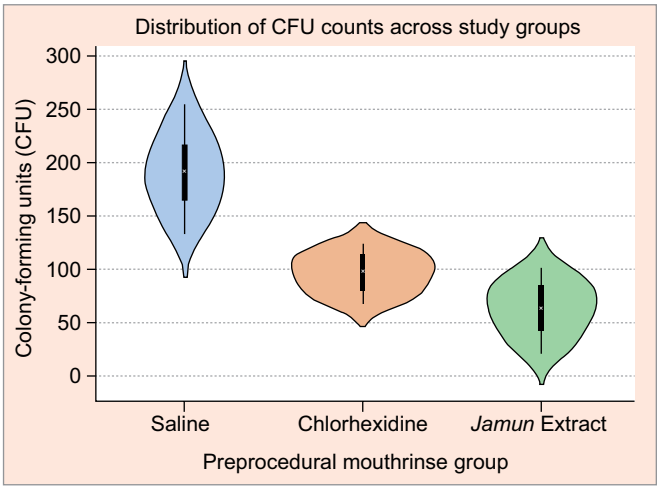

Fig. 2: Distribution of CFUs across study groups

Levene's test demonstrated no significant difference in variance across groups ($p = 0.421$), supporting the suitability of parametric statistical analysis (Table 1).

Intergroup Comparison

A one-way ANOVA revealed a statistically significant difference in microbial aerosol burden among the three groups ($p < 0.001$). The data suggest that the choice of preprocedural mouthrinse influences bacterial contamination levels during scaling.

The calculated effect size ($\eta^2 = 0.412$) suggested that approximately 41.2% of the observed variation in microbial counts could be attributed to the intervention employed, indicating a substantial treatment effect.

Games-Howell post hoc analysis demonstrated significant differences between the saline group and both antimicrobial rinse groups. Compared with saline, chlorhexidine was associated with lower microbial counts (mean difference = 0.48021, $p < 0.001$), while *S. cumini* seed extract showed a greater reduction in bacterial recovery (mean difference = 0.71234, $p < 0.001$).

A statistically significant difference was also observed between the chlorhexidine and *S. cumini* groups (mean difference = 0.23213, $p = 0.015$). Under the conditions of the present study, the *S. cumini* formulation demonstrated lower mean CFU values than chlorhexidine, suggesting enhanced suppression of aerosol-associated bacterial contamination (Table 2). The distribution of log-transformed CFU counts among the study groups is presented in Figure 2. The graphical pattern was consistent with the statistical findings, demonstrating progressively lower microbial counts from the saline group to the chlorhexidine group and subsequently to the *S. cumini* seed extract group.

The study demonstrated that the preprocedural mouthrinse with *S. cumini* seed extract substantially reduced bacterial contamination levels during scaling compared with the normal saline rinse. This finding suggests that the seed extract can be used as an alternative natural method for infection control and for reducing bacterial contamination during dental practice.

Discussion

The present randomized controlled clinical trial evaluated the effect of *S. cumini* seed extract as a preprocedural oral rinse on microbial aerosol recovery generated during ultrasonic scaling. The findings demonstrated that the type of rinse used before instrumentation influenced the quantity of recoverable microorganisms. Among the evaluated groups, participants who received *S. cumini* seed extract showed the lowest mean transformed CFU values, followed by chlorhexidine and normal saline. These observations suggest that the bioactive components present in *S. cumini* may contribute to modifying the oral microbial load before aerosol-generating dental procedures.[6,7]

Aerosol dissemination during ultrasonic scaling continues to be an important concern in dental infection control due to the release of microorganisms originating from saliva, gingival tissues, and dental biofilm.[1] Ultrasonic instrumentation generates aerosol particles through the interaction between high-frequency vibrations and irrigation fluid, which may result in microbial spread into the clinical environment.[14] A recent systematic review and meta-analysis by Gonçalves Lomardo et al. highlighted the potential contamination risk associated with ultrasonic scalers and emphasized the importance of additional preventive strategies to reduce microbial dissemination during aerosol-generating procedures.[15] The findings of the present study support this approach, as the use of antimicrobial rinses resulted in lower microbial recovery compared with saline.

In this study, chlorhexidine demonstrated reduced aerosol-associated bacterial recovery compared with normal saline. This finding may be explained by the established antimicrobial action of chlorhexidine, which involves disruption of bacterial cell membrane integrity and leakage of intracellular components.[4,5] Chlorhexidine is widely studied as a preprocedural mouthrinse due to its broad antimicrobial activity and substantivity.[4] Recent clinical investigations continue to report decreased bacterial aerosol recovery following the use of chlorhexidine-based rinses before dental procedures.[16] However, concerns associated with prolonged use, including tooth discoloration, taste alteration, and mucosal irritation, have encouraged the evaluation of alternative agents with improved biological acceptability.[4,5]

The lower microbial recovery observed in the *S. cumini* seed extract group may be related to the presence of multiple phytochemical constituents, including tannins, flavonoids, phenolic compounds, and anthocyanins.[6,7] These compounds have demonstrated antimicrobial activity through various mechanisms, such as alteration of bacterial membrane permeability, interference with microbial adhesion, and inhibition of bacterial growth.[7,8] Unlike single-target antimicrobial agents, plant-derived compounds may exert combined effects through multiple biochemical pathways, which may contribute to their observed microbial inhibitory potential.[17]

The antimicrobial potential of *S. cumini* has been reported in previous investigations. Chismirina et al. demonstrated inhibitory activity of *S. cumini*-based formulations against cariogenic microorganisms, suggesting its possible application in oral microbial control.[13] Similarly, Pandey et al. reported antibacterial activity of *S. cumini* seed extract against oral pathogens, supporting the biological relevance of the present findings.[8] Recent experimental evidence has further demonstrated that *S. cumini* seed-derived compounds exhibit antimicrobial and antioxidant properties, strengthening their potential as a natural therapeutic agent.[17] A study conducted by Nugraheni et al. demonstrated that *S. cumini* seed extract in a mouthwash showed antibacterial properties against periodontopathogens, which is consistent with the current study.[18]

The present results are also in agreement with the increasing evidence supporting herbal-based oral rinses in dental practice. Bernardo et al. reported that herbal mouthrinse formulations exhibited measurable antimicrobial activity against oral microorganisms and may serve as potential alternatives or adjuncts to conventional chemical agents.[19] Similarly, recent clinical evaluations of botanical oral formulations have suggested that plant-derived rinses may provide microbial reduction while maintaining favorable safety profiles.[20] These findings support further investigation of *S. cumini* as a naturally derived approach for microbial control during dental procedures.

Although the findings indicate a promising role of *S. cumini* seed extract, this extract may reduce the microbial burden after ultrasonic scaling and may be used as a plant-based natural adjunct to control microbial contamination in routine dental practice. The limitations of the present study are that it was conducted with a relatively limited sample size and assessed only the immediate reduction of aerosol-associated microorganisms following a single ultrasonic scaling procedure. Microbial assessment was performed using total CFU counts without identification of individual microbial species, with a short incubation period, which restricts the generalizability of the results. Additionally, the persistence of antimicrobial activity following rinsing was not evaluated. Further investigations involving multicenter trials, molecular microbial analysis, a longer incubation period, and evaluation of substantivity are required to determine its long-term clinical effectiveness.

Overall, the present study demonstrates that *S. cumini* seed extract has potential as a plant-derived preprocedural rinse for reducing microbial aerosol recovery during ultrasonic scaling. The observed antimicrobial effect, together with its natural origin and phytochemical properties, supports further exploration of this formulation as an adjunctive strategy for infection control in dental practice.

Conclusion

S. cumini seed extract mouthrinse demonstrated a reduction in bacterial aerosol contamination generated during ultrasonic scaling. The antimicrobial activity observed with *S. cumini* was comparable to 0.2% chlorhexidine, indicating its potential role as an herbal preprocedural rinse in dental practice. Further studies with larger sample sizes and long-term evaluation are required to confirm its effectiveness and clinical application in infection control.

Clinical Significance

The use of Syzygium cumini seed extract mouthrinse may provide a natural approach for reducing microbial aerosols during ultrasonic scaling procedures and may support infection control practices in dental settings.

Ethical Approval

Ethical approval was obtained from the Institutional Ethics Committee (IEC No: VDCW/IEC/167/2019).

Data Availability

The data analysed during the current study are available from the corresponding author on request, subject to institutional approval and participants' consent.

Acknowledgments

This study was supported by the Indian Council of Medical Research under the Short-term Studentship Programme. The authors declare no conflicts of interest related to this study.

Author's Contribution

EG Priyadharshini conceptualized the study, protocol, data collection, and statistical analysis. DC Manjula contributed to data collection and patient recruitment. Vaishnavi PS performed statistical analysis. Preethi P contributed to manuscript drafting, and T Kavin and Rudhra K critically reviewed the manuscript.

All authors read and approved the manuscript.

AI Declaration

No AI tool was used in the preparation of the manuscript.

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