Original Article

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Dentistry Section

Efficacy of Neem and Cinnamon Extracts in Reducing Bacterial Contamination during Scaling: A Randomised Clinical Study

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ABSTRACT

Introduction: Ultrasonic scaling involves the production of a high amount of aerosols, which combine with oral microbes and become a potential source of airborne contaminants. Chemical antimicrobial agents like Chlorhexidine (CHX) (the gold standard) are known to reduce microbial contamination in dental waterlines. However, natural extracts are gaining popularity as an economical yet effective means of reducing bacterial load.

Aim: To compare the efficacy of neem and cinnamon extracts in reducing bacterial contamination during scaling.

Materials and Methods: A single-centered, parallel multiple-arm, randomised clinical study was conducted at the Department of Periodontics and Oral Implantology, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India from September 2019 to March 2020. Scaling was performed for patients in all four groups (n=128, 32 each group) - Water, 0.2% Chlorhexidine, Neem, and Cinnamon Extract. Two blood agar petri dishes were placed on either side of the patient at a distance of 40 cm from the patient's head, and one blood agar petri dish was placed on the operator's chest. The agar plates were then incubated aerobically for 48 hours for Colony Forming Units (CFU) count. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) and Tukey's posthoc test.

Results: Cinnamon showed a 25.2% reduction in CFUs, whereas neem showed an 11.7% reduction compared to distilled water. However, chlorhexidine had the maximum bacterial reduction. The agar plates at the chest position had the highest bacterial growth, while the left position had comparatively fewer microbial colonies.

Conclusion: Chlorhexidine exhibited the highest percentage of CFU reduction. However, when used as a Dental Unit Water Line (DUWL) coolant, herbal extracts such as cinnamon and neem caused a significant reduction in CFUs compared to distilled water, with cinnamon performing better than neem. Therefore, herbal extracts like neem and cinnamon can be considered cost-effective alternatives for minimising aerosol contamination in DUWL.

Keywords: Aerosol contamination, Dental unit water lines, Herbal extracts

INTRODUCTION

Aerosol contamination is an inevitable event happening in dental clinics, regardless of advanced equipment being used in recent times. These aerosolised droplets formed during dental procedures remain suspended in the air for a considerable duration, and inhalation of such droplets aids in acute and chronic respiratory problems, which can be even fatal [1,2]. The hazards of such droplets have been proven in the recent pandemic Coronavirus Disease-2019 (COVID-19) due to the presence and detectability of COVID-19 in infected patients' saliva, which was alarming during an aerosol-generating dental treatment [3].

Dental waterline is known to harbour numerous microorganisms due to the stagnation of water. The bacterial load of such untreated Dental Unit Waterlines (DUWLs) can usually exceed 105 CFU/mL [4]. Coolant from these pipelines can always be a source of aerosol contamination during cavity preparation and scaling [5]. Antimicrobial coolants act on the innate microflora, thereby minimising the hazards of any aerosols produced during dental treatment.

Among several compounds, Chlorhexidine is a well-documented antimicrobial agent with a bacteriostatic action at low concentration and bactericidal at high concentration [6]. However, herbal extracts such as neem, cinnamon, turmeric, aloevera, tulsi, clove, peppermint, green tea, and many more are gaining attention due to their phytochemical components, which can be antimicrobial and anti-inflammatory with negligible alcohol content, making them tissue-friendly [7].

A recent study comparing the efficacy of cinnamon, CHX with distilled water has revealed that herbal extracts can be a beneficial yet economical alternative to distilled water [8]. Likewise, neem, being a wonder herb, is enriched with many potentially useful phytochemical ingredients that have both antibacterial and antiviral properties [9,10]. There is currently no scientific evidence to support the beneficial effects of neem in DUWL. Hence, the present study aims to compare the potential benefits that could be obtained by replacing the gold standard CHX with low-cost yet beneficial herbal extracts such as neem and cinnamon in minimising the bacterial load in aerosols produced during regular dental procedures.

MATERIALS AND METHODS

The current study was a single-centered, parallel multiple-arm randomised clinical study conducted in the Department of Periodontics and Oral Implantology, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India, from September 2019 to March 2020. Institutional Ethical Clearance was obtained (SRMU/M&HS/SRMDC/PG/009), and it was registered in the Clinical Trial Registry India (CTRI/2021/09/036416).

Participants were explained about the procedure. Verbal and written informed consent was obtained from all study participants. The recruited participants were systemically healthy and screened for the gingival index.

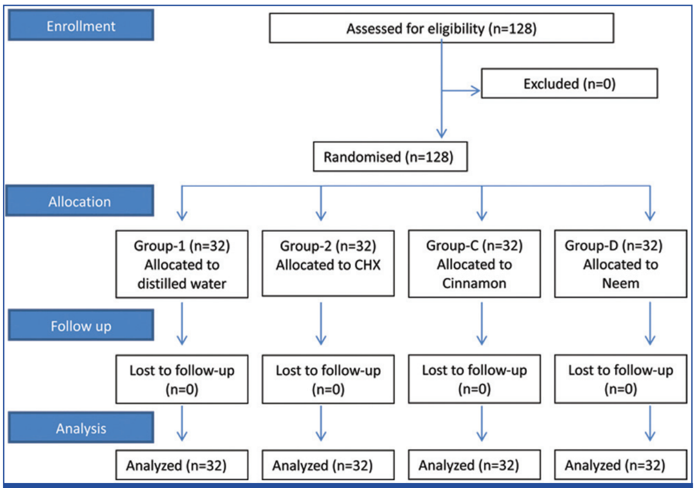
Inclusion criteria: Those with moderate to severe gingivitis (GI score of 1.5-3), having a minimum of 20 permanent teeth, and indicated for full-mouth scaling in a single visit were included in the study.

Exclusion criteria: Patients with any systemic illness, smokers, pregnant women, and those who had undergone oral prophylaxis or antibiotic therapy in the last three months were excluded from the study.

Sample size calculation: The sample size was calculated based on $n=Z^2 \times Sd^2/L^2$ {n-sample size, Z-Confidence Interval (1.96), Sd-Standard Deviation (74.803) [8], L-Level of Precision (5%)} as 128, with 32 participants per group, and the power of the study fixed at 80%.

Study Procedure

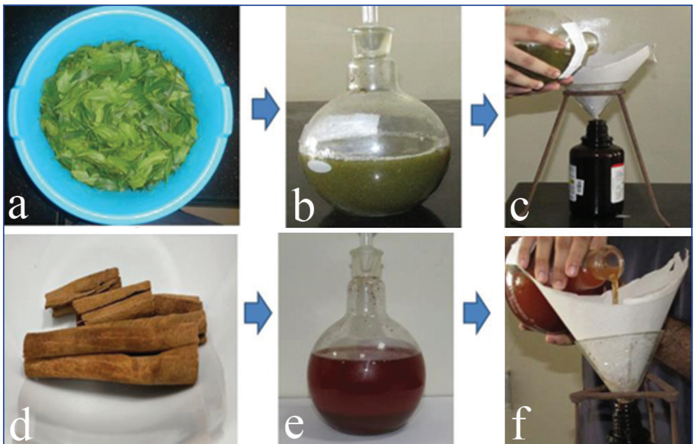
The patients were selected based on a simple random sampling method and were allocated into four groups. Group 1 received ultrasonic scaling with distilled water, Group 2 received ultrasonic scaling with 0.2% Chlorhexidine, Group 3 received ultrasonic scaling with cinnamon extract, and Group 4 received ultrasonic scaling using neem extract [Table/Fig-1].



[Table/Fig-1]: Consolidated Standards of Reporting Trials (CONSORT) flowchart.

Preparation of chlorhexidine: Lavage regularly after removal of the agar plates. A commercially available 0.2% CHX mouthwash was procured and dispensed in water in a 1:1 ratio prior to the beginning of the ultrasonic scaling.

Preparation of neem extract: Neem leaves (Azadirachta indica) were procured and authenticated by the botanist. The leaves were shadow dried for 14 days and then sent to the laboratory for preparation of the aqueous extract by the Soxhlet extraction method [11]. The Minimum Inhibitory Concentration (MIC) of neem leaf extract was set at 500 µg/mL, and based on this, the final concentration of the aqueous neem extract was adjusted to 0.5 g/L [Table/Fig-2] [11].



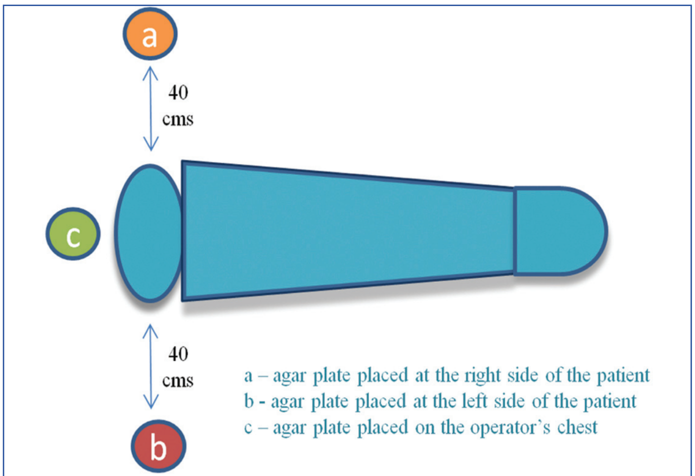
[Table/Fig-2]: a) Raw neem leaves; b) Neem extract prepared by Soxhlet extraction method; c) Filtration of neem extract; d) Raw cinnamon; e) Cinnamon extract prepared by Soxhlet extraction method; f) Filtration of cinnamon extract.

Preparation of cinnamon extract: Commercially available cinnamon (Cinnamomum verum) was purchased and authenticated by the botanist. The product was sent to the laboratory for preparation of the aqueous extract by the Soxhlet extraction method [12]. The MIC of cinnamon extract was set at 4 mg/mL, and based on this, the final concentration of the aqueous cinnamon extract was adjusted to 4 g/L [Table/Fig-2] [12].

Method of performing the study: Ultrasonic scaling was performed for all the subjects who met the inclusion criteria in a closed dental clinic. Prior to the procedure, an agar plate was placed in the center of the closed operatory room where the scaling was supposed to be performed for 20 minutes. It was then sent for microbiological quality testing to determine adequate quality control and to ensure that the operatory was not contaminated with airborne pathogens. This agar plate was not sent for statistical analysis as it was meant for quality control purposes.

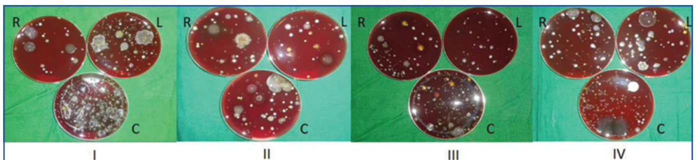
The patients were randomly assigned to one of the four groups: Water/CHX/Neem/Cinnamon group. Prior to scaling, the fresh aqueous extracts were dispensed in the Dental Unit Waterlines (DUWLs) by adjusting the amount of water to be added to the concentrated extracts for the patients undergoing scaling in the neem and cinnamon group. Scaling was performed for a period of approximately 15 minutes using an ultrasonic scaler and suction during the procedure.

Use of agar plates: Commercially available 20% Sheep Blood agar plates (Himedia Inc. India) were used in the present study. Three agar plates were used for each subject - two agar plates were placed at a distance of 40 cm on either side of the patient's head, and one agar plate was placed at the chest of the operator prior to the commencement of scaling [Table/Fig-3]. After completion of the 15-minute procedure, the agar plates were sealed and transported to the microbiology laboratory. If oral prophylaxis was not completed within the 15 minutes, the procedure was carried out regularly after removal of the agar plates.



[Table/Fig-3]: Illustration of the agar plates placed during scaling.

Microbial analysis: The agar plates were incubated in an aerobic chamber for a period of 48 hours. Colonies of bacteria were counted using a semi-digital colony counting device and denoted as the number of CFUs visualised on the agar plates [Table/Fig-4].



[Table/Fig-4]: Colony Forming Units (CFU) formed on blood agar plates. I- Water group, II-CHX group; III-Cinnamon group; IV-Neem group.

STATISTICAL ANALYSIS

The results were statistically analysed for the CFUs and the gingival index using Statistical Package for Social Sciences (SPSS) software (IBM SPSS Statistics for Windows, version 23.0, Armonk, NY: IBM

Corp. Released 2018). One-way ANOVA was performed to evaluate the CFUs in different groups, whereas Tukey's posthoc test was done for pair-wise comparison. Any data with a p-value less than 0.05 was considered statistically significant.

RESULTS

The age and gingival index of the patients enrolled in the study for the four groups were comparable (p>0.05) as shown in [Table/Fig-5]. [Table/Fig-6] shows the CFUs that were recorded in all four study groups in all three positions, along with the comparison of the difference in CFUs among the study groups. The lowest CFUs were found in the CHX group in the chest position. The difference was significant when compared with water and cinnamon (p-value <0.001). There were also significantly fewer CFUs in the cinnamon group compared to water and neem with p-values of <0.05 and <0.001, respectively. In the right position, the comparison between chlorhexidine and all the other groups showed a statistically significant difference with p-value <0.05. In the left position, there were significantly fewer CFUs in the CHX group compared to water and neem with a p-values of <0.001 and <0.001, respectively, as shown in [Table/Fig-6]. No significant difference in CFUs was seen between the cinnamon and neem groups in any of the positions (p>0.05). The average mean percentage of CFU reduction was highest in the Chlorhexidine group at 69.4% compared to distilled water. The neem and cinnamon groups had 11.7% and 25.2% average mean percentage reduction, respectively, compared to distilled water, as shown in [Table/Fig-7].

Variables	Water	CHX	Cinnamon	Neem	p-value
Age	28.40±8.11	28.53±9.81	25.12±5.27	28.03±9.53	0.679
GI	2.03±0.19	2.18±0.19	2.30±0.19	2.30±0.16	0.235

[Table/Fig-5]: Descriptive statistics of all the four study groups (N=32/group, expressed in mean±SD).

Positions	Water	CHX	Cinnamon	Neem	Significance	p-value
Chest	141.00± 73.32	25.62± 25.40	93.81± 45.11	116.59± 58.26	Water vs CHX 115.37±13.37	<0.001*
					Water vs Cinnamon 47.18±13.37	0.003*
					Water vs Neem 24.40±13.37	0.267
					CHX vs Cinnamon -68.18±13.37	<0.001*
					CHX vs Neem -90.96±13.37	<0.001*
					Cinnamon vs Neem 22.78±13.37	0.327
Right	91.93± 74.4	38.34± 45.11	104.31± 57.46	83.78± 47.62	Water vs CHX 53.59±14.33	0.002*
					Water vs Cinnamon -12.37±14.33	0.824
					Water vs Neem 8.15±14.33	0.941
					CHX vs Cinnamon -45.43±14.33	0.010
					CHX vs Neem -45.43±14.33	0.010
					Cinnamon vs Neem 20.53±14.33	0.482
Left	97.28± 75.26	31.09± 27.06	69.40± 40.31	88.75± 56.50	Water vs CHX 66.18±13.23	<0.001*
					Water vs Cinnamon 27.87±13.23	0.157
					Water vs Neem 8.53±13.23	0.917
					CHX vs Cinnamon -38.31±13.23	0.023
					CHX vs Neem -57.65±13.23	<0.001*
					Cinnamon vs Neem 19.34±13.23	0.464

[Table/Fig-6]: CFU (/mL) counted on blood agar plates during aerobic analysis (expressed in mean difference and significance).
*p-value <0.05-statistically significant

Groups	Mean% reduction CFU chest	Mean% reduction CFU left	Mean% reduction CFU right	Average mean% reduction
Distilled Water	0	0	0	0
Chlorhexidine	81.8%	68%	58.3%	69.4%
Neem	17.3%	8.8%	8.9%	11.7%
Cinnamon	33.5%	28.7%	13.5%	25.2%

[Table/Fig-7]: Mean percentage reduction in CFUs in comparison to the percentage of CFUs in distilled water.

DISCUSSION

The basic procedures that are executed for almost all patients everyday cause the dental environment to be highly exposed to contagious airborne particles, which are primarily produced during oral prophylaxis by mechanised scalers, tooth preparation using an air rotor, air abrasive devices, and water air syringes. Coolant for such procedures is obtained from the DUWL, which, when combined with saliva, generates aerosols that are contaminated with microbes, thereby posing a significant risk factor for transmitting infectious diseases, sometimes with fatalities [13]. Research has shown that the use of an antimicrobial coolant can reduce the microbial load on dental personnel [14]. Chemical agents such as Chlorhexidine and Povidone have substantiated their efficacy in reducing aerosol contamination, however, cinnamon is the only herbal extract that has been researched as a DUWL coolant [8,15].

In the present study, commercially available CHX mouthwash (Dr. Reddy's) has been used in the DUWL at a concentration of 0.1% [16]. However, while CHX is the gold standard in the synthetic group due to its effective antibacterial effect, it has a number of negative effects, including staining, altered taste perception, tissue irritation, and the rare occurrence of parotid swelling [17].

Many herbs and spices are known for their antioxidant and antimicrobial properties. They are available in different forms, such as extracts, essential oils, pastes, and powders. In the present study, cinnamon and neem are taken into consideration due to their common availability and high antibacterial efficacy. Cinnamon is a well known spice that contains several phytochemicals, such as cinnamaldehyde, cinnamyl acetate, eugenol, and cinnamyl alcohol. The benefits of cinnamaldehyde against various perio-odontogenic bacteria have been proven [18,19]. When used in appropriate concentrations, cinnamon acts as a potent antimicrobial agent. Cinnamon extracts damage the cell membrane, alter the lipid profile, inhibit cell division, and exhibit anti-quorum sensing effects, contributing to its mode of action against gram negative and gram-positive bacteria [20]. Neem exhibits similar properties.

Neem is a potent antibacterial agent due to the presence of gallotannin. It is also known to have a direct antiviral effect against various viruses [9]. The mechanism of action of neem as an antimicrobial is through cell wall lysis, and it also shows microbial growth inhibition [10]. Numerous studies highlight the benefits of neem in controlling bacterial counts when used as a preprocedural mouthrinse. Additionally, neem has shown substantial effects against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus causing COVID-19 [21,22].

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of any extract that acts against a specific microorganism. In the present study, the MIC of Neem extract (A.indica) was set at 500 µg/mL (0.5 g/L), which was calculated based on the MIC of neem leaves against E.faecalis, a bacterium known to have 35% colonisation in the microbiota of periodontitis patients [11,23,24]. The MIC for cinnamon extract against P.gingivalis (a keystone pathogen) was determined to be 3.12±0.65 mg/mL. For ease of extract processing, this value was rounded up to 4 mg/mL (4 g/L) [12].

In the present study, the CHX group showed the maximum reduction in CFUs, around 69.4%, when compared to distilled water. However, the herbal extracts, cinnamon and neem, also demonstrated a reduction of 25.2% and 11.7% in the number of CFUs, respectively, compared to distilled water. A significant and comparable decrease in CFUs was observed in both the neem and cinnamon groups when compared to the CFUs obtained in the distilled water group. This suggests that natural products like neem and cinnamon can be used as alternatives to distilled water. Similarly, Mamajiwala AS et al., evaluated the efficacy of cinnamon, CHX, and distilled water in Dental Unit Water Lines (DUWL), and found that cinnamon and CHX performed better than distilled water (p-value <0.005) [8].

Gupta D and Jain A, compared the effectiveness of cinnamon, CHX, and distilled water as a mouth rinses in terms of reducing plaque formation and gingival inflammation. They concluded that cinnamon had greater benefits compared to distilled water [25]. Similarly, Jalaluddin M et al., conducted a study comparing neem extract with CHX as a mouthwash and found that both neem extract and CHX had better clinical outcomes with reduced plaque and gingival indices [26]. The antiplaque property of Neem toothpaste was substantiated by Sugiarta AP and Lessang R, where significant reductions in plaque and papillary bleeding indices were observed compared to a placebo [27].

The increasing prevalence of multiresistant strains of microorganisms has become a significant concern in modern medicine. In this context, herbal products have gained attention and preference due to their potential antimicrobial properties. These products contain a diverse range of phytochemicals, including alkaloids, tannins, essential oils, and flavonoids, which have been found to exhibit pronounced antimicrobial activity [28]. However, it is crucial to acknowledge that while herbal products show promise as antimicrobial agents, they are not a panacea. Their efficacy, safety, and standardisation may vary, and more research is needed to establish their effectiveness against specific oral conditions. Patient acceptance when using herbal extracts in terms of palatability, provoking any gag reflex, and the freshness felt post-treatment are also concerns that depend on the concentration of the extract. Herbal extracts may pose health hazards if used in very high concentrations. Therefore, striking the right balance between the Minimum Inhibitory Concentration (MIC) and toxicity would make these herbal extracts a promising alternative to distilled water. In the present study, CFUs were analysed for aerobic bacteria only; however, both aerobic and anaerobic microbes are equally present in the aerosol [29].

Limitation(s)

The MICs of the herbal extracts were not calculated in the present study. Calculating the MICs of the herbal extracts against the periodontal pathogens before commencing the study could have provided more substantial and informative results. Determining the MICs would have allowed for a better understanding of the precise concentration required to effectively inhibit the growth of oral microorganisms, which would have been a valuable step to enhance scientific rigor and clinical applicability. Further studies can be done considering the anaerobic bacteria with a larger sample size to validate the efficacy of herbal extracts.

CONCLUSION(S)

The results of the present study indicate that chlorhexidine was the most effective in reducing CFUs, followed by cinnamon extract, while neem extract was the least effective. These findings suggest that natural remedies can be just as beneficial as the latest advancements in oral healthcare. This article also highlights the fact that herbal products can be equally effective in combating oral microorganisms. By emphasising the potential advantages of neem and cinnamon over distilled water, our study establishes that herbal extracts, which are easily prepared and readily available, present a viable alternative to distilled water for minimising aerosol contamination.

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