



Anti-Plaque and Anti-Gingivitis Efficacy of 0.25% Lemongrass Oil and 0.2% Chlorhexidine Mouthwash in Children

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ABSTRACT

Objectives: Research is ongoing to find safe and effective oral hygiene aids for oral self-care in children. Mouthwashes are used to complete the process of mechanical plaque control. Lack of affordability and side effects of most commercially available mouthwashes limit their use in children. Hence, the cost-effective and easily available essential oil, lemongrass oil, when formulated as a mouthwash, may possibly serve as an adjunct to oral hygiene maintenance. The main objective of this study was to compare the efficacy of lemongrass oil and chlorhexidine (CHX) mouthwash in children.

Materials and Methods: Sixty healthy children between 9-12 years were selected. During the initial visit, the plaque pH, plaque index (PI), and gingival index (GI) were assessed, and oral prophylaxis was performed. The patients were randomized into three groups (n=20) and received 0.25% lemongrass oil mouthwash (group A), 0.2% CHX mouthwash (group B), and oral prophylaxis alone (group C). The patients were recalled after 14 and 21 days. ANOVA with post-hoc Bonferroni and paired t-test were used to analyze the results by SPSS software.

Results: Intragroup comparison of PI and GI showed a significant decrease between 14 and 21 days in groups A and B ($P \leq 0.05$). Intragroup comparison of the mean plaque pH in group A showed a significant increase at day 21 compared with baseline ($P = 0.028$).

Conclusion: The results showed that the lemongrass oil mouthwash was effective in reducing PI and GI in children. Thus, it may be used as a good herbal alternative to CHX mouthwash.

Keywords: Gingivitis; Chlorhexidine; Mouthwashes; Lemongrass Oil; Dental Plaque

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INTRODUCTION

Gingivitis and periodontitis are generally thought of as diseases of adulthood. Pediatric dentists and general practitioners have traditionally paid little attention to the gingival and periodontal health status of children [1]. Gingival inflammation without detectable alveolar bone loss or clinical attachment loss is common in children [2], and shows a significantly higher incidence compared to dental caries in the pediatric population [1]. Gingivitis affects more than 70% of children older than 7 years of age [3]. Mechanical plaque

removal is the most effective way for prevention of caries and periodontal disease [1]. Although mechanical plaque control can provide excellent results, many pediatric patients are unwilling, unable, or untrained to practice routine effective mechanical plaque removal. These facts necessitate chemical plaque control as an adjunct to mechanical plaque control to maximize the efficacy of oral hygiene practice [4]. Chlorhexidine (CHX), as the gold standard antimicrobial agent, is the most widely used mouthwash. Its ability to attach to hard and

soft tissues in the oral cavity is responsible for its substantivity for a long period after its application. However, taste alteration, oral mucosal ulceration, unilateral/bilateral parotid swelling, brown discoloration of dentition, restorative materials, and dorsum of the tongue, and enhanced supra-gingival calculus formation have been reported as the side effects of long-term CHX use [5]. CHX also has documented effects on vital tissues, such as alteration of mitochondrial activity, cytotoxicity for the periodontal ligament cells, and inhibition of protein synthesis [6]. An effective substitute to CHX with all its benefits and fewer side effects has long been awaited, and is highly recommended.

The useful bioactivities of essential oils from plant sources particularly antimicrobial activity have formed a basis for the development of new alternative remedies/therapeutics as an alternative to chemical formulations for prevention and inhibition of human pathogens [7]. *Cymbopogon citratus*, commonly known as lemongrass, represents an important species among the Poaceae family [8], and possesses a strong lemony odor due to its high content of aldehyde citral. Many scientific studies have provided evidence on its antimicrobial, antioxidant, antifungal, anti-inflammatory, and superoxide scavenging properties of lemongrass in several disease models, and have reported its growth inhibitory effects on periodontopathic pathogens [8,9]. Based on the abovementioned therapeutic properties, lemongrass essential oil mouthwashes have been formulated. To date, no study is available on this topic and this is among the pioneer studies regarding the use of lemongrass oil mouthwash in children. The main study objective is to compare and evaluate the plaque control effectiveness of 0.25% Lemongrass oil and 0.2% CHX mouthwash.

MATERIALS AND METHODS

This study was conducted at the Department of Pediatric and Preventive Dentistry, Government Dental College, Bangalore on healthy, cooperative children between 9-12 years who were selected from residential institutions, and had similar food habits for the purpose of standardization. Ethical clearance

for conduction of the study was obtained from the institution ethical committee (Ref no: GDCRI/IEC-ACM(2)/5/2017-18).

The sample size was calculated to be 20 in each group (total: 60), considering $\alpha=0.05$, effect size of 0.25, and power of 95%. Inclusion criteria consisted of patients with mild to moderate gingival inflammation (scores 1-2) with healthy periodontium aged between 9-12 years, compliant children (Frankl's behavior rating scale 3 or 4) capable of maintaining oral hygiene by themselves, and children without orthodontic appliances and prosthesis. Participants were excluded if they were uncooperative (Frankl's behavior rating scale 1 or 2), required special health care needs, took antibiotics for systemic diseases or had a history of using topical fluoride, mouthwash, or xylitol chewing gums in the past 4 weeks, had systemic diseases or conditions affecting saliva secretion, reported any known allergy to lemongrass oil, or had active carious- or intraoral-lesions.

Children were randomized into three groups by the lottery method. The test groups evaluated were:

- Group A: Lemongrass oil (organic *Cymbopogon citratus*; Hippocrates health institute, USA): 20 patients were randomly assigned to this group and received 0.25% lemongrass oil mouthwash to be used for the duration of 21 days (Fig. 1).
- Group B: CHX (Rexidin from Warren, Indoco Remedies Ltd, Mumbai, India): 20 patients were randomly assigned to this group and received 0.2% CHX mouthwash to be used for the duration of 21 days (Fig. 1)



Fig. 1: Lemongrass oil (Hippocrates health institute, USA) and Chlorhexidine mouthwash (Rexidin, Warren, Indoco Remedies Ltd, Mumbai, India)

• Group C:- Oral prophylaxis: 20 patients were randomly assigned to this group and only oral prophylaxis was performed for them.

Formulation of 0.25% lemongrass oil mouthwash:

The lemongrass oil mouthwash was formulated at the Department of Pharmacy, Government Pharmacy College, Bangalore. The lemongrass oil mouthwash was prepared from lemongrass essential oil using the cosolvency approach [10]. For this purpose, 100ml solution was formulated by adding 0.275ml lemongrass oil to 5.882ml of 75% ethyl alcohol, as lemongrass oil is water-insoluble. The volume was reached to 100ml with purified water, and stirred for 30min. Next, 1g of pure talc powder was added as an adsorbent and stirred for 30min. It was then filtered using the Whatman filter papers (55mm 1441-055, Sunshine Instruments, Coimbatore, India). The solution was allowed to rest at room temperature for 3 months, and the pH was measured to be 6.4.

Procedure:

On the test day, a plaque sample of approximately 1mg was collected from the buccal and interproximal surfaces of the posterior teeth with a sterile blunt explorer (GDC, Hoshiarpur, India) (Fig. 2). The collection time of the samples was standardized to be 30s.



Fig. 2: Plaque collection using a blunt probe

The plaque sample was thoroughly mixed with 20ml of distilled water in a beaker to assess the baseline plaque pH using an automated digital pH meter (pHep, Hanna instruments, India) (Fig. 3).

The electrode was calibrated with distilled water every time before each measurement to keep the pH at 7. Next, it was placed in the beaker for 30s to measure the pH of the plaque



Fig. 3: Measuring the plaque pH by a digital pH meter

[11]. This was followed by the assessment of plaque index (PI) [12] and gingival index (GI) [13] and oral prophylaxis using hand scalers to set the plaque score at zero. Finally, 0.25% lemongrass oil mouthwash and 0.2% CHX mouthwash were given to groups A and B, respectively. Group C did not receive any mouthwash.

Children were instructed to use 10ml of the mouthwash twice daily for 1min, after brushing, in the morning before breakfast, and at night after dinner for 21 days. The mouthwashes had to be dispensed in the given plastic cup, and the patients were asked to swish for 1 min and then spit. They were asked not to eat or drink anything for 30min after using the mouthwash. For accurate measurements, the participants were instructed not to take any food or beverage within 2h prior to the procedure.

During the course of the study, to maintain the oral hygiene practice similar, children were given similar toothbrushes and toothpastes, and the modified Bass tooth brushing technique was instructed to them, and the parents were asked to fill out the compliance form. The parents/guardians were instructed to supervise the toothbrushing and the proper use of the mouth rinse on a daily basis and were instructed to report back any unpleasant experience associated with the use of the mouthwash. All children were recalled at 14 days to record their GI and PI. Final assessment was done at 21 days to evaluate the plaque pH, PI and GI. The findings were recorded by a single examiner, who was

trained and calibrated to record the PI and GI, at all the intervals and for all the groups. CHX and lemongrass oil mouthwash were given in identical bottles for the purpose of blinding of the participants. The Cohen's Kappa statistic was used to test intra-examiner reliability for the assessment of plaque pH, PI and GI ($k = 0.8-0.9$) using ANOVA with the post-hoc Bonferroni test, paired t-test and repeated measures ANOVA. The collected data were analyzed using SPSS version 22.

RESULTS

Of the 20 children in each group, there were 9 males and 11 females in group A (mean age, 10.56 years), 8 males and 12 females in group B (mean age, 9.86 years) and 10 males and 10 females in group C (mean age 10.76 years).

The mean differences in plaque pH, PI and GI scores were compared among the groups using ANOVA with post-hoc Bonferroni and reanalyzed using repeated measure ANOVA.

Based on our results, the three groups were not significantly different at baseline or 21 days regarding the mean plaque pH with the respective P-values of 0.80 and 0.19 (Table 1). The plaque pH significantly decreased in group A (-0.08) at 21 days compared with baseline ($P=0.028$); whereas, no significant change was noted in groups B (-0.04, $P=0.36$) and C (0.03, $P=0.55$) in this aspect.

The PI score of the three groups at days 1, 14, and 21 did not show any statistically significant differences (Table 2), but the mean GI was significantly different among the three groups at days 14 and 21 (Table 3).

Table 1: Intergroup comparison of plaque pH at baseline and 21 days using ANOVA (n=20)

Time	Groups	Minimum	Maximum	Mean	Standard deviation	F-value	P
Day 1	A	6.8	7.9	7.35	0.32	0.22	0.8
	B	6.9	7.9	7.29	0.27		
	C	6.8	8	7.31	0.28		
Day 21	A	6.9	7.9	7.43	0.29	1.71	0.19
	B	6.9	7.7	7.34	0.22		
	C	6.7	7.8	7.28	0.28		

Table 2: Inter-group comparison of PI scores at days 1, 14, and 21 using ANOVA (n=20)

Time	Groups	Minimum	Maximum	Mean	Standard deviation	F-value	P
Day 1	A	0.8	1.8	1.23	0.3	0.75	0.47
	B	0.7	1.8	1.26	0.29		
	C	0.8	1.6	1.15	0.24		
Day 14	A	0.6	1.6	1.05	0.28	0.35	0.7
	B	0.5	1.5	1.12	0.27		
	C	0.7	1.5	1.06	0.22		
Day 21	A	0.6	1.4	.92	0.24	2.69	0.07
	B	0.5	1.6	1.06	0.26		
	C	0.6	1.5	1.1	0.24		

Table 3: Intergroup comparison of GI at 1, 14, and 21 days using ANOVA (n=20)

Time	Groups	Minimum	Maximum	Mean	Standard deviation	F value	P
Day 1	A	0.6	1.8	1.34	0.33	0.54	0.58
	B	0.8	1.9	1.38	0.29		
	C	0.9	1.9	1.45	0.34		
Day 14	A	0.6	1.5	1.11	0.26	4.18	0.02*
	B	0.7	1.7	1.23	0.26		
	C	0.8	1.8	1.37	0.31		
Day 21	A	0.6	1.2	0.98	0.19	13.26	0.001*
	B	0.6	1.6	1.11	0.25		
	C	0.8	1.8	1.41	0.33		

*Significant

The mean change in GI score was significant in all groups and at baseline, and 14 and 21 days (Table 4).

Table 4: Intragroup changes in the mean PI and GI scores at different time points using repeated measures ANOVA

Index	Group	Repeated measures	P
PI	A	96.59	0.001*
	B	45.45	0.002*
	C	12.58	0.005*
GI	A	63.95	0.001*
	B	70.67	0.001*
	C	7.47	0.004*

*Significant

Table 5 shows intragroup pairwise comparison of PI and GI between the three different time points. Statistically significant differences were noted between all time-points in groups A and B in PI. In group C, significant differences were noted only between days 1 and 14, and days 1 and 21 in PI. Regarding the GI score, statistically significant differences were noted between all time-points in groups A and B, but no significant difference was noted in group C.

DISCUSSION

High prevalence of gingivitis points to the inefficiency of self-performed mechanical plaque control procedures. Also, it has been proven that the prevention of gingivitis and periodontitis depends on adequate supra-gingival plaque control [14,15]. Although CHX is one of the most widely used mouthwashes, it has certain disadvantages [16].

Hence, in recent times, there has been an increase in demand for alternative medicine.

Lemongrass oil is an essential oil with antidepressant, antioxidant, antiseptic, bactericidal, fungicidal, astringent, nervine, and sedative properties [17].

The baseline mean plaque pH assessed for the lemongrass oil mouthwash, CHX mouthwash, and oral prophylaxis groups was found to be 7.35, 7.29 and 7.31, respectively. These values were close to the resting plaque pH which is described to be within the range of 6-7 [18]. The assessed plaque pH in our study was in accordance with the study by Muralikrishnan et al, [19] who assessed the baseline plaque pH to be 7.59 in 8-12-year-old children. The assessed baseline plaque pH in our study was slightly higher than the rate reported by Garg et al, [18] and Talreja et al, [20] who assessed the baseline plaque pH after the consumption of various food items by children and reported it to be in the range of 5.9-6.6 and 6.1-6.2, respectively. The plaque pH after the intervention using the mouthwash experienced an increase compared with baseline in groups A and B. But there was a reduction in plaque pH in group C, which might be because of the lack of additional chemical measures. Even though the raise in plaque pH was not statistically significant, this increase in pH levels could be attributed to the use of mouthwash. The intragroup comparison showed a statistically significant (P=0.028) rise in plaque pH between baseline and 21 days only in the lemongrass oil mouthwash group. This shows the superior efficacy of lemongrass oil for plaque pH maintenance in children compared with CHX.

Table 5: Intragroup comparison of PI and GI using the post-hoc Bonferroni test

PI	Day 1 versus day 14		Day 1 versus day 21		Day 14 versus day 21	
	Mean difference	P	Mean difference	P	Mean difference	P
A	0.18	<0.001*	0.31	<0.001*	0.13	0.001*
B	0.14	<0.001*	0.195	<0.001*	0.055	0.036*
C	0.09	<0.001*	0.055	0.013*	-0.035	0.208
GI	Day 1 versus Day 14		Day 1 versus Day 21		Day 14 versus Day 21	
	Mean difference	P	Mean difference	P	Mean difference	P
A	0.235	<0.001*	0.36	<0.001*	0.125	0.001*
B	0.145	<0.001*	0.27	<0.001*	0.125	0.002*
C	0.08	0.013	0.04	0.05	-0.04	0.217

*Significant

The lemongrass oil mouthwash was effective in inhibiting the plaque regrowth. It can penetrate into the plaque biofilm, kill the pathogenic microorganisms by disrupting their cell wall, and inhibit their enzymatic activity according to Kukkamalla et al [21]. This may explain the rise in plaque pH with the use of lemongrass oil mouthwash in the present study. The rise in plaque pH in the CHX group was in accordance with the literature whereby it acts by damaging the cell membrane of prokaryotes and by disrupting the cytoplasmic components [22].

Intergroup comparison of PI score at baseline, and 14 and 21 days showed no statistically significant difference. But there was a reduction in the mean PI in each group at 21 days compared with baseline, and the PI score at all assessed time points remained within the range of 0.1-1.9 (fair). Statistically significant reduction in the mean PI at different time points compared with baseline was noted in all three groups. This finding is in accordance with the results of Dany et al, [23] in adults who showed a significant change in the mean PI score at both 14 ($P=0.019$) and 21 ($P=0.017$) days. This could be because of the motivation, and proper brushing technique which could have influenced the removal of dental plaque on a regular basis and prevention of plaque accumulation on the teeth. Pairwise intragroup comparison of time points showed statistically significant differences in groups A and B. Both mouthwash groups showed greater reduction in PI score compared with the oral prophylaxis group which may be due to better outcome of chemical plaque control as an adjunct to mechanical plaque control. Comparing the two mouthwash groups, the results showed similarities between 0.25% lemongrass oil mouthwash and 0.2% CHX mouthwash. The anti-biofilm activity of the lemongrass oil mouthwash can be due to the presence of various constituents such as citral, limonene, citronellal, β -myrcene, linalool and geraniol [21].

An in vitro study by Kukkamalla et al, [21] on lemongrass oil showed it to be an effective antiplaque agent at both 0.5% and 0.25% concentrations in the mouthwash form, and it was more effective than CHX. The viscosity of

the oil inhibits bacterial adhesion and plaque co-aggregation. In 0.2% CHX mouthwash group, reduction in PI score is due to the supporting evidence that CHX attacks the bacterial cell membrane, causing leakage or precipitation of cellular contents as well as its specificity property. It can bind to bacteria and salivary mucin, and prevent their absorption and inhibit plaque colonization on the teeth as such [23].

The present results showed a significant reduction in GI in all three groups at 14 and 21 days. This can be explained as, with elimination of the main etiologic factor, i.e. dental plaque, a subsequent reduction in GI score occurs. The highest reduction in the mean GI score was recorded with the use of 0.25% lemongrass oil mouthwash and was found to be 0.98 ± 0.19 . The anti-oxidant and anti-inflammatory properties of lemongrass oil might have contributed to this finding, and this finding is in accordance with that of Dany et al, [23] in adults. In gingivitis, the inflammatory infiltrates consist of neutrophils, lymphocytes, and plasma cells which affect the oxidative stress and anti-oxidant pattern of the tissues. Natural antioxidants in the lemongrass oil can overcome this and maintain homeostasis [23]. Citral in lemongrass oil acts by induction of the glutathione antioxidant pathway and subsides oxidative stress due to stereoisomer, neral, and geraniol in its composition.

It also donates hydrogen to free radicals and terminates the chain reaction of lipid metabolism. Flavonoid, another chemical component of lemongrass oil, has many biological activities and is involved in antioxidant activity [24]. Such substantial evidence supports the superior antioxidant and anti-inflammatory properties of lemongrass oil, which might be responsible for the reduction in GI score in the lemongrass oil mouthwash group in the present study.

Comparison of the mean PI and GI among the studied mouthwash groups revealed a significant difference, which indicates the comparable efficacy of the mouthwashes used in this study for maintenance of gingival health. In an in vivo study, Biswas et al. [22] evaluated the anti-plaque and anti-gingivitis activity of

CHX and an herbal mouth rinse and found both of them to be equally effective. Such studies support the efficacy of CHX mouthwash in children. The results of Biswas et al. [22] were similar to the findings in CHX group in our study. The results of the present study on children who used lemongrass mouthwash cannot be directly compared with similar studies because to the best of our knowledge, it is one of the pioneer studies, evaluating the effect of 0.25% lemongrass essential oil as a mouthwash on dental plaque and gingivitis in children. It might be more effective than CHX in prevention of dental caries as well. Further studies are recommended on this topic.

CONCLUSION

The results of the present study showed that both mouthwashes were effective in improvement of PI and GI. Also, 0.25% lemongrass oil mouthwash was found to be comparable to 0.2% CHX in reduction of gingivitis and as an antiplaque agent. Lemongrass oil mouthwash showed better results in the plaque pH scores in children than CHX. Thus, lemongrass oil mouthwash may be more beneficial for plaque control and gingival health, and it may be suitable for use as an alternative to CHX as an adjunct to mechanical plaque control.

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CONFLICT OF INTEREST STATEMENT

None declared.

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