

Comparative Evaluation of 0.25% Lemongrass Oil Mouthwash and 0.2% Chlorhexidine Mouthwash in Fixed Orthodontic Patients Suffering from Gingivitis

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ABSTRACT

Aim: The aim of this study is to compare the antiplaque and antigingivitis efficacy of 0.25% lemongrass oil mouthwash and 0.2% chlorhexidine mouthwash in patients undergoing fixed orthodontic treatment, who are suffering from gingivitis.

Materials and methods: A total of 60 patients undergoing fixed orthodontic treatment with mild-to-moderate gingivitis were selected for the study. The patients were randomly divided into three groups of twenty each, that is, group I: 0.25% lemongrass oil mouthwash ($n = 20$); group II: 0.2% chlorhexidine mouthwash ($n = 20$); and group III: oral prophylaxis ($n = 20$). Baseline gingival index (GI) and plaque index (PI) were assessed followed by oral prophylaxis was done and the PI score was set to zero for all the patients. Patients were asked to swish their mouth with their respective mouthwashes and brushing (twice daily), that is, morning and before bedtime for 21 days. The PI and GI scores were recorded for all three groups on the 14th and the 21st days. The *post hoc* Bonferroni test was used for multiple comparisons of mean differences among variables after the application of the analysis of variance (ANOVA) test for comparison within the groups.

Results: A lower PI and the GI were found in the lemongrass oil mouthwash group by the 14th and the 21st days, respectively, a statistically significant difference ($p < 0.001$) compared to the chlorhexidine mouthwash group.

Conclusion: The findings of the current study suggested that 0.25% lemongrass oil mouthwash has the potential to be used as a natural or herbal alternative to chlorhexidine mouthwash.

Clinical significance: Besides, orthodontic appliances increase the retention

sites and complicate the process of efficient oral care procedures. Combining those factors in the orthodontic process usually leads to higher dental plaque retention and gingival inflammation. Apart from the amount of dental plaque, the microbial composition may also change significantly during the orthodontic treatment.

Fixed orthodontic appliance placement often leads to a higher incidence of gingivitis in a patient. White spot lesion on the labial or lingual surface of banded or bonded teeth during fixed orthodontic treatment has long been recognized as a major concern.² The use of orthodontic appliances such as bands, brackets, ligature wires, and elastics inside the patient's mouth could set off a negative impact on oral cavity status that may lead to an increase in acid-producing bacteria and accumulation of food particles that will cause plaque formation.^{2,3} The plaque that accumulates in an oral environment of a fixed orthodontic patient is rather more cariogenic as an increase in the lactobacilli population causes a surge in the concentration of carbohydrate per milligram plaque formed. Failure to manage plaque may lead to demineralization, loss of tooth support, and an increased number of carious lesions.

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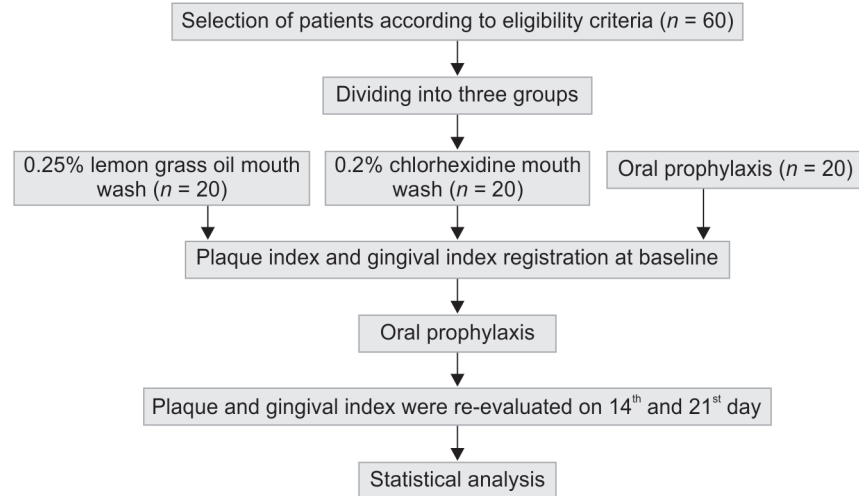
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Greater accumulation of plaque at the gingival margin could be associated with a higher incidence and severity of gingivitis.⁴ Although in most cases, gingivitis may remain stable during orthodontic treatment, in some cases, gingivitis may advance to periodontitis which could result in an irremediable loss of tooth-supporting tissues.⁵ Hence, monitoring and maintaining periodontal and gingival health status during orthodontic

Flowchart 1: Flowchart representing the study design

treatment and persistent reinforcement of satisfactory oral hygiene routines have become an essential part of modern orthodontics.

Dental plaque control can be achieved in two ways. First, by physical methods that mainly involve tooth brushing, interproximal brushing, flossing, and irrigation; second, by chemical methods.⁶ In the latter, accumulation of plaque can be controlled chemically by the use of mouth rinse.⁷ Mouth rinses are considered efficient antiplaque and antigingivitis agents and 0.2% chlorhexidine gluconate mouthwash is considered a gold standard.

Mouthwash should be used as an adjunct to brushing in the presence of fixed orthodontic appliances. Numerous studies have demonstrated the efficacy of chlorhexidine-based mouthwashes in patients with fixed orthodontic appliances.⁸⁻¹¹ The main advantage of 0.2% chlorhexidine gluconate is an important therapeutic agent in controlling the *Streptococcus mutans* and lactobacilli levels of orthodontic patients with fixed appliances. However, prolonged usage of chlorhexidine-based mouthwash has been reported to be associated with a number of local side effects such as brown discoloration of teeth, paresthesia, oral mucosal ulcerations, tongue in the form of black hairy tongue, taste perturbation, etc.¹²⁻¹⁴ The upsurge in the prevalence of its side effects has encouraged scientists to research herbal agents as complementary and alternative. A growing number of consumers are embracing the philosophy that herbal-based products are better for their health and the environment.⁵

Essential oil mouthwash such as Lemongrass oil with antibacterial activity can be considered as it prevents bacterial aggregation and thus it slows their multiplication and extracts the bacterial endotoxins.^{15,16} Essential lemongrass oil is extracted from lemongrass that belongs to the *Gramineae* family. Lemongrass oil has an abundance of medicinal use such as antifungal, antibacterial, antiseptic, antioxidant, analgesic, antipyretic, anti-inflammatory, astringent, carminative properties, and its proven antifungal and antibacterial properties, and its effectiveness can be equated to that of penicillin.¹⁷

The phytochemical constituents of lemongrass are the herb *Cymbopogon*, a member of the *Poaceae* family, which is also referred to as lemongrass. A perennial grass, lemongrass can reach a height of 1 m, and has multiple stiff, green stalks that emerge from short and rhizomatous roots. A perennial aromatic grass known as *Cymbopogon citratus* is widely grown in the Philippines

and Indonesia. It is also cultivated in America and Asia, and, more specifically, in their tropical regions. The plant is mostly an indigenous herb to India, but it is also grown in several tropical and subtropical nations, including Pakistan.^{16,17}

Hence, considering the side effects caused by prolonged use of chlorhexidine gluconate-based mouthwash and the trust people have in natural herbal products such as lemongrass oil, the present study was designed to compare the antiplaque and antigingivitis efficacy of 0.25% lemongrass oil mouthwash with that of 0.2% chlorhexidine mouthwash in fixed orthodontic patients suffering from gingivitis.

MATERIALS AND METHODS

The present study was carried out in the Department of Orthodontics and Dentofacial Orthopedics, Santosh Dental College and Hospital, Ghaziabad. The study was approved by the institutional ethical committee and review board (SU/2016/431/33). The ethical committee of Santosh University approved the protocol and all of the subjects signed an ethical committee consent form agreeing to serve for the study. This was carried out over a period, from June 2015 to January 2017.

Study Population

A total number of 60 subjects, who were undergoing fixed orthodontic treatment at Santosh Dental College and Hospital, Ghaziabad, Uttar Pradesh, India, were included in the study. All 60 subjects had all teeth bonded and bands on the first molars.

Study Design

This comparative clinical trial was mainly done to check the efficacy of 0.25% lemongrass oil and 0.2% chlorhexidine gluconate mouthwash in orthodontics patients suffering from mild-to-moderate gingivitis (Flowchart 1).

Inclusion Criteria

- Patients undergoing fixed orthodontic treatment with fully-bonded metal brackets (MBT-0.022" slot) on their teeth and bands on their molars.
- Aged 13 years or above.
- Had mild-to-moderate gingivitis.

Exclusion Criteria

- Patients with systematic diseases.
- Have any specifically known allergy to essential oil or lemongrass oil.
- Known hypersensitivity to chlorhexidine.
- Pregnant and nursing patients.
- Patients who were unable to complement the follow-up requirements of the study.
- Patients using antibiotics or underwent antibiotic treatment 2 months prior to study.
- Smokers.
- Have a habit of any form of tobacco chewing.
- Patients having any oral disease like a periodontal abscess or any perio–endo lesions.

All 60 subjects were randomly grouped into three groups:

- Group I: 0.25% lemongrass oil mouthwash (*n* = 20)
- Group II: 0.2% chlorhexidine mouthwash (*n* = 20)
- Group III: Oral prophylaxis (*n* = 20)

Following standard protocols, 0.25% lemongrass oil mouthwash was prepared in the Department of Pharmacology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India. A few lemongrass stalks were taken then the outer layer was removed, and then crushed coarsely with a mortar and pestle. This was infused in a jar of base oil (olive oil) and was kept in a warm, sunny place for 48 hours, while commercially available 0.2% chlorhexidine gluconate mouthwash (Hexidine®) was used (Fig. 1).

The oral examination was carried out by a single calibrated examiner, who was blind to the allocation status of the participants in the Department of Public Health Dentistry, Santosh Dental College and Hospital, Ghaziabad, Uttar Pradesh, India. The same examiner assessed the participants again at the end of the experiment.

Baseline plaque index (PI) and gingival index (GI) were measured on both the labial and lingual aspects of maxillary and mandibular teeth, which was followed by oral prophylaxis at the same visit, and PI score was set to zero. Gingival status was recorded through GI by Loe and Silness,¹⁸ similarly, dental plaque status was recorded through PI by Silness and Loe.¹⁹

Modified bass brushing technique along with interdental brushing was demonstrated to participants.²⁰ All the participants were briefed by the examiner on the use of mouthwash and brushing. Patients belonging to lemongrass oil and chlorhexidine gluconate mouthwash groups were instructed to regularly use their respective types of mouthwash for 1 minute and brush twice daily, that is, morning and before bedtime for 21 days (Figs 2A and B) whereas patients on oral prophylaxis were advised for brushing twice a day for 2–3 minutes for 21 days (Fig. 2C). All subjects were



Fig. 1: Container showing 0.25% lemongrass oil mouthwash



A



B



C

Figs 2A to C: (A) Patient advised 0.25% lemongrass oil mouthwash; (B) Patient advised 0.2% chlorhexidine mouthwash; (C) Patient advised oral prophylaxis

Table 1: Difference in PI score on labial surface from day 1 to day 14 and from day 1 to 21

	Difference in PI score (labial surface) from day 1 to day 14				Difference in PI score (labial surface) from day 1 to day 21			
	Mean	Standard deviation	F-value	p-value	Mean	Standard deviation	F-value	p-value
Lemongrass	0.29	0.12			0.53	0.15		
Chlorhexidine	0.34	0.17	20.538	< 0.001*	0.56	0.22	19.457	<0.001*
Oral prophylaxis	0.10	0.04			0.25	0.12		

*p-value is less than 0.05

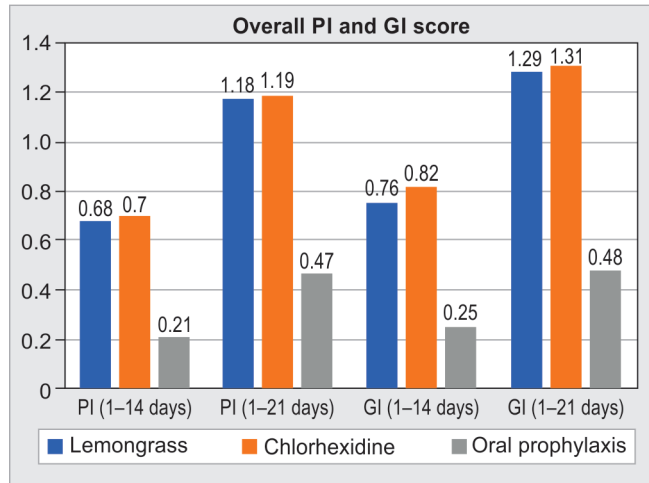


Fig. 3: Statistically significant reduction in PI and GI scores on the 14th and 21st days in all the three groups, that is, lemongrass oil, chlorhexidine gluconate, and oral prophylaxis groups

Table 2: Difference in PI score on lingual/palatal surface from day 1 to day 14 and from day 1 to 21

	Difference in PI score (lingual/palatal surface) from day 1 to day 14				Difference in PI score (lingual/palatal surface) from day 1 to day 21			
	Mean	Standard deviation	F-value	p-value	Mean	Standard deviation	F-value	p-value
Lemongrass	0.39	0.12			0.65	0.16		
Chlorhexidine	0.36	0.13	47.197	<0.001*	0.63	0.11	93.305	<0.001*
Oral prophylaxis	0.10	0.03			0.21	0.05		

given a new orthodontic toothbrush, the interdental brush of identical make and brand along with the same toothpaste to sustain equilibrium throughout the experimental period.

To keep a check on patient compliance, regular performance was fortified every 3–4 days through telephonic calls. On the 14th and 21st days, participants were accessed again for PI and GI scores as the time required for the development of gingivitis ranges from 10 to 21 days.²¹

The PI and GI were evaluated on all the teeth in the patient’s mouth excluding third molars. Both the PI and GI were evaluated in two gingival units of the same teeth (labial/buccal and palatal/lingual).

In addition to these clinical parameters, participants were also interviewed at their follow-up examination for any adverse effect or symptom experienced such as taste modification, injury to the oral mucosa, burning sensation, or inflammation during the use of mouthwash.

After each cycle, that is, on the 14th and 21st days, the patients brought a filled questionnaire showing their compliance.

The data obtained were subjected to statistical analysis. One-way analysis of variance (ANOVA) test was done for comparison

within the groups. *Post hoc* Bonferroni test was done after ANOVA for multiple comparisons. One-way ANOVA compares the means of two or more independent groups in order to determine whether there is statistical evidence that the associated population means are significantly different. One-way ANOVA is a parametric test. Software XLSTAT was used in this study.

RESULTS

The results of our present study showed a statistically significant reduction in PI and GI score on both the 14th and 21st days in all the three groups, that is, lemongrass oil, chlorhexidine gluconate, and oral prophylaxis (Fig. 3).

When both types of mouthwash were compared for PI score, 0.2% chlorhexidine mouthwash showed a slightly better result as compared to 0.25% lemongrass oil mouthwash on the labial and buccal surfaces on day 1 to day 14 and day 1 to day 21 by 0.05 and 0.03 (Table 1), while lingual and palatal surfaces 0.25% lemongrass oil mouthwash showed slightly better results on the 14th and 21st days by 0.03 and 0.02, respectively (Table 2). On comparing both types of mouthwashes for GI score for labial surface on the days

Table 3: Difference in GI score on labial surface from day 1 to day 14 and from day 1 to 21

	Difference in GI score (labial surface) from day 1 to day 14				Difference in GI score (labial surface) from day 1 to day 21			
	Mean	Standard deviation	F-value	p-value	Mean	Standard deviation	F-value	p-value
Lemongrass	0.43	0.21			0.69	0.28		
Chlorhexidine	0.44	0.24	19.272	<0.001*	0.66	0.29	21.432	<0.001*
Oral prophylaxis	0.12	0.05			0.24	0.09		

Table 4: Difference in GI score on lingual/palatal surface from day 1 to day 14 and from day 1 to 21

	Difference in GI score (lingual/palatal surface) from day 1 to day 14				Difference in GI score (lingual/palatal surface) from day 1 to day 21			
	Mean	Standard deviation	F-value	p-value	Mean	Standard deviation	F-value	p-value
Lemongrass	0.33	0.16			0.60	0.27		
Chlorhexidine	0.38	0.18	13.802	<0.001*	0.65	0.24	20.489	<0.001*
Oral prophylaxis	0.13	0.14			0.24	0.13		

Table 5: Difference in overall PI score from day 1 to 14 and from day 1 to 21

	Difference in PI (overall score) from day 1 to day 14				Difference in PI (overall score) from day 1 to day 21			
	Mean	Standard deviation	F-value	p-value	Mean	Standard deviation	F-value	p-value
Lemongrass	0.68	0.19			1.18	0.26		
Chlorhexidine	0.70	0.24	48.296	<0.001*	1.19	0.26	67.810	<0.001*
Oral prophylaxis	0.21	0.05			0.47	0.14		

Table 6: Difference in overall GI score from day 1 to 14 and from day 1 to 21

	Difference in GI (overall score) from day 1 to day 14				Difference in GI (overall score) from day 1 to day 21			
	Mean	Standard deviation	F-value	p-value	Mean	Standard deviation	F-value	p-value
Lemongrass	0.76	0.26			1.29	0.40		
Chlorhexidine	0.82	0.31	32.765	<0.001*	1.31	0.38	40.461	<0.001*
Oral prophylaxis	0.25	0.13			0.48	0.16		

1–14, 0.2% chlorhexidine mouthwash showed a better result by 0.01 than 0.25% lemongrass oil mouthwash (Table 3). The mean difference in GI score for the labial surface from the day 1 to day 21 showed better results for 0.25% lemongrass oil mouthwash by 0.03 as compared to 0.2% chlorhexidine mouthwash (Table 3). The mean difference in GI score for lingual/palatal surface, 0.2% chlorhexidine mouth wash showed to be more effective than 0.25% lemongrass oil mouthwash by 0.05 on both range of days from day 1 to day 14 and day 1 to day 21 (Table 4).

The combined score for both the surfaces for PI showed that 0.2% chlorhexidine mouthwash was marginally better than 0.25% lemongrass oil mouthwash by 0.02 and 0.01 on both the 14th and 21st days, respectively (Table 5).

In our study, the mean PI score for 0.2% chlorhexidine mouth wash for the overall surface on the 14th and 21st days is 0.70 ± 0.24 and 1.19 ± 0.26 , respectively, while the mean PI for the overall surface for 0.25% lemongrass oil mouthwash is found to be 0.68 ± 0.19 and 1.18 ± 0.26 on the 14th and 21st days (Table 5).

The combined score for both the surfaces for GI score concluded that 0.2% chlorhexidine mouth wash was marginally better than 0.25% lemongrass oil mouth wash by 0.06 and 0.02 on both the 14th and 21st days (Table 6).

In our study, the mean GI score for 0.2% chlorhexidine mouth wash for the overall surface on the 14th and 21st days is 0.82 ± 0.31 and 1.31 ± 0.38 , respectively, while the mean GI score for the overall surface for 0.25% lemongrass oil mouthwash is found to be 0.76 ± 0.26 and 1.29 ± 0.40 on the 14th and 21st days (Table 6). In our study, we could suggest that 0.25% lemongrass oil mouthwash may be a good herbal alternative to mouthwash containing 0.2% chlorhexidine gluconate.

DISCUSSION

Recently, essential oils have started gaining popularity. The antibacterial and nontoxic properties of essential oils make them uniquely suitable for use in oral care products. Essential oil mouthwashes have demonstrated equal effectiveness in inhibiting plaque regrowth compared to chlorhexidine mouthwashes because they penetrate the plaque biofilm and disrupt the cell wall of the pathogenic microbes within it and inhibit their enzymatic activity.^{20,21} One of these essential mouthwashes, that is, lemongrass oil-based mouthwash has been used in this study. Lemongrass oil has an abundance of medicinal use such as antifungal, antibacterial, antiseptic, antioxidant, analgesic, antipyretic, anti-inflammatory,

astringent, carminative properties, and its proven antifungal and antibacterial properties and its effectiveness can be equated to that of penicillin. It has shown minimal side effects and are affordable to the general population.¹²⁻¹⁷ In this study, we compared the efficacy of mouthwashes containing 0.25% Lemongrass oil and 0.2% chlorhexidine and oral prophylaxis as a controlled group in inhibiting plaque accumulation and gingivitis in orthodontic patients with fixed braces. The control groups were selected to offset the Hawthorne effect.²²

Plaque accumulation is a naturally occurring process wherein bacteria interact with acquired salivary pellicles which develop over the tooth surface shortly after brushing. It is often the main reason for gingivitis.^{18,23,24} Gingivitis often progresses into periodontitis in susceptible subjects.⁵

Thus, dental plaque plays a major factor in the commencement as well as the advancement of gingival and periodontal diseases. A direct connection has been established between the severity of gingival inflammation and plaque levels. Usually, if an antimicrobial mouth rinse is added to the daily oral hygiene routine of patients undergoing fixed orthodontic treatment, they typically show remarkable improvements in their oral hygiene status.¹⁹ The benefits of chlorhexidine mouthwash are even more significant in orthodontic patients, as it reduces plaque accumulation and gingivitis.¹⁰

Numerous studies have shown the efficiency of chlorhexidine-based mouthwashes in patients with fixed orthodontic appliances. It has been shown that the use of 0.2% chlorhexidine gluconate for mouth rinse in fixed orthodontic patients substantially reduces the amount of plaque-causing bacterial colonies.^{8,9} Anderson et al.¹⁰ compared the short-term clinical effect of 0.12% chlorhexidine gluconate-based mouthwash and placebo mouth rinses in 30 adolescent patients aged between 11 years and 15 years undergoing orthodontic treatment and concluded that using chlorhexidine gluconate mouthwash in addition to regular oral hygiene practices, was efficient in reduction of plaque and gingivitis in adolescents undergoing orthodontics.

Nevertheless, the study by McCoy et al.²⁵ showed that chronic use of chlorhexidine could be associated with side effects such as loss of taste, discoloration of dental surfaces, burning sensations, or mucosal irritation.

All three groups experienced a statistically significant reduction in PI score on both the 14th and 21st days in the present study. It can be ascribed that several constituents of lemongrass oil, including citral, citronellal, D-limonene, Geraniol, and linalool lemongrass oil has an antibiofilm activity.²⁶ By penetrating between the fatty acyl chains that make up membrane lipid bilayers, terpenes present in lemongrass oil alter cellular permeability, alter membrane fluidity and disrupt lipid packing. Surface and morphological changes were caused by these phenomena thus reducing the ability for oral pathogens to adhere to the surface of the tooth.²⁷ The presence of these agents might help in preventing biofilm-associated infections since adhesion is a defining feature of biofilm formation.²⁸

According to the results, PI score reductions were more pronounced in the mouthwash groups than in the oral prophylaxis-only groups. This result may be explained by chemical plaque control is a better adjunct to mechanical plaque control.²⁵ As compared to 0.25% lemongrass oil mouthwash; 0.2% chlorhexidine mouthwash was marginally superior as far as PI score is concerned. A critical reason for this reduction could be that chlorhexidine attacks the bacterial cell membrane, thereby causing leakage and

precipitation of the contents of the cells; and by binding to the mucins in saliva, it inhibits plaque formation and pellicle formation. Additionally, it binds to bacteria and prevents them from adhering to the teeth.²⁹ A study conducted by Dany et al.¹² showed a slightly better picture for the 0.25% lemongrass oil mouthwash as compared to 0.2% chlorhexidine mouthwash regarding PI score. Kukkamalla et al.¹⁷ study showed that lemongrass oil at both 0.5 and 0.25% concentration in mouthwash is a more effective antiplaque agent than that chlorhexidine.

All three groups also demonstrated statistically significant improvement in GI scores both on 14th and 21st days. Dental plaque is associated with gingivitis, therefore, eliminating the principal etiological factor will reduce GI scores.³⁰ In the 0.25% lemongrass oil mouthwash group, the antioxidant and anti-inflammatory properties of Lemongrass oils contributed to the reduction in GI score. Han and Parker³¹ found that Lemongrass essential oil showed anti-inflammatory effects in pre-inflamed human dermal fibroblasts. Boukhatem et al.³² indicated that lemongrass (*C. citratus*) essential oil possesses the promising potential for the development of drugs for treating skin inflammation and fungal infections.

In addition to enhancing the clinical resolution of gingival inflammation, lemongrass oil has an antimicrobial effect that prevents the recolonization of periodontal pockets with bacteria. Furthermore, the antioxidant activity of the same prevents periodontal tissue destruction and promotes healing.³³ Satthanakul et al.³⁴ demonstrated that lemongrass oil has powerful antimicrobial properties against oral malodor-causing microbes and significantly reduces halitosis. Thus, lemongrass oil mouth rinse could be an alternative mouth rinse choice for the prevention of gum diseases, malodor, and plaque accumulation.

The use of a 0.25% lemongrass oil mouthwash has been shown to have a pragmatic effect in inhibiting gingivitis and plaque accumulation in patients undergoing fixed orthodontic treatment and the findings in the present study affirm the same.

CONCLUSION

From the outcomes of the present study, it can be concluded that the active ingredients of 0.25% lemongrass oil mouthwash containing citral, citronellol, D-limonene, geraniol, and linalool helped in inhibiting plaque and gingivitis on both the 14th and 21st days in orthodontic patients undergoing fixed bracket therapy. It also showed antimicrobial, anti-inflammatory, and antioxidant properties. It can be suggested that 0.25% lemongrass oil mouthwash may be a good herbal alternative to mouthwash containing 0.2% chlorhexidine gluconate.

There have been very few studies on using lemongrass oil as an active agent in mouthwash; therefore, further research with different parameters is necessary in order to find out more about the potential benefits and negative effects of its use, if any.

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