LEMONGRASS OIL MOUTHWASH AND ITS ANTIPLAQUE EFFICACY: AN IN-VITRO STUDY USING SPECTROPHOTOMETER
Kukkamalla Meena Anand¹, Pentapathi Kalyan Chakravarthi², Yadav Muditi Krishna³, Rao Ram Mohan⁴, Kamath Shobha⁵
1. Associate Professor, Department of Periodontology, Manipal College of Dental Sciences, Manipal University Manipal
2. Department of Periodontology, Manipal College of Dental Sciences, Manipal University, Manipal
3. Department of Periodontology, Manipal College of Dental Sciences, Manipal University, Manipal
4. Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal
5. Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal

ABSTRACT: Considering the uses of lemongrass oil, the aim of the present study was to find out the antiplaque property of lemongrass oil mouthwash in vitro. Pooled saliva was collected in a sterile container with the polymethymethacrylate strips (1cm breadth X 4.5cm height) which were of the size of the slot of spectrophotometer, was kept in the containers, and incubated at 37degree centigrade for 48 hours. The strips were stained with erythrosine dye for 30seconds and rinsed in water and kept in spectrophotometer and value was recorded. The same procedure was done for 10 strips to rule out the bias. The second ten strips were taken stained, rinsed with water, rinsed with the lemongrass oil mouthwash 0.25% with alcohol, rinsed, stained with erythrosine, rinsed with water and readings were taken from the spectrophotometer. The same procedure was done for lemongrass oil mouthwash 0.5% with alcohol, lemongrass oil mouthwash 0.25% and 0.5% without alcohol and chlorhexidine mouthwash, with and without alcohol. The results showed that the lemongrass oil mouthwash at 0.5% concentration showed reduction in the plaque as that of chlorhexidine. The present study concluded that the lemongrass oil mouthwash can be used as an adjunct to mechanical oral hygiene.

Key words: lemongrass oil mouthwash, chlorhexidine mouthwash, spectrophotometer

INTRODUCTION: Dental plaque is defined as a highly specific variable structural entity formed by sequential colonization of microorganisms on the tooth surface, epithelium & restorations. It is also defined as the soft deposits that form the biofilm adhering to the tooth surface or other hard surfaces in the oral cavity including removable and fixed restoration. Dental plaque is found above the gingival margin and in direct contact with the gingival margin referred to as marginal plaque. Sub-gingival plaque is found below the gingival margin, between the tooth and gingival Sulcular tissues.

Dental plaque accumulation is the pre requisite for the development of gingivitis (Loe et al 1965). Gingivitis may develop into periodontitis in susceptible individuals and prevention of gingivitis is successful in prevention of periodontitis. Since both gingivitis and
periodontitis are plaque associated oral conditions the removal of dental plaque should inhibit their occurrence and progression.

Potential removal of supra gingival bacterial plaque by means of tooth brush remains the most widely accepted method of oral disease prevention. Continuation of effective personal oral hygiene regimens requires a well-motivated patient who uses device in a proper fashion for sufficient duration of time and with adequate frequency.

Chemical control of plaque is considered to be adjunct to mechanical oral hygiene practices, agents being most commonly used in the form of tooth paste and mouth rinse. Chlorhexidine digluconate is to date most thoroughly studied and most effective antiplaque and anti-gingivitis agent when addressing oral hygiene (Gjermo 1989).[2] However several side effects associated with its use like staining of teeth and restorations, unpalatable taste etc have stimulated the search for new alternatives.

Essential oils are ideal for use in oral care products because they are both antibacterial and non-toxic – a rare combination. Lemongrass oil is important essential oil, extracted from Lemon grass which belongs to the section of Andropogan called Cymbopogam of the family Germineae. The botanical genus name Cymbopogon for lemongrass is derived from Greek 'cyombo' boat and 'pogon' beard. It refers to the bulbous end which is boat-shaped and the long blade-like green leaves resembling a beard.

Lemongrass oil has plethora of medicinal uses. It is said to have anti-bacterial[3], anti-fungal[4], anti-oxidant[5], anti-inflammatory[6] properties. The effectiveness of lemongrass oil is based mainly on its centuries-old reputation as a folk remedy.

Considering the various uses of lemongrass oil an attempt was made to harness its properties, aim of the study was to evaluate antiplaque efficacy of lemongrass oil mouthwash and to compare with that of chlorhexidine mouth wash.

**MATERIALS AND METHODS:**

The study was done in department of Periodontology in collaboration with department of biochemistry. The study was approved by the ethical committee of the Institution. Pooled saliva was collected from the volunteers in a sterile container with the polymethyl methacrylate strips (1cm breadth X 4.5cm height). The polymethyl methacrylate strips were cut in the size of the slot of the spectrophotometer. The strips were kept in the pooled saliva up to the three fourth of the length of the strips. They were incubated at 37degree centigrade which is the, approximate temperature of the oral cavity, for 48 hours in the incubator.

Lemongrass oil mouth wash was prepared with 0.25% and 0.5% with and without alcohol indigenously in department of Pharmacology, Manipal.
University. Chlorhexidine mouthwash was procured from the pharmacy over the counter.

The strips were divided into 6 groups. Each group had 10 strips. Group 1- lemongrass oil 0.25% alcohol mouthwash, group 2- lemongrass oil mouthwash 0.25% without alcohol, Group 3- lemongrass oil 0.5% alcohol mouthwash, Group 4-lemongrass oil mouthwash 0.5% without alcohol, Group 5- chlorhexidine alcohol mouthwash 0.2%, Group 6- chlorhexidine mouthwash 0.2% without alcohol.

The spectrophotometer was set at 530nm as the lambda max of erythrosine range between 525 -530 nm. Group 1 strips were stained with erythrosine, washed in distilled water, observed in spectrophotometer and the value was recorded. Each strip was rinsed in lemongrass oil mouthwash 0.25% with alcohol for 30 seconds. The strip was stained with erythrosine and rinsed in distilled water and the readings were recorded using spectrophotometer. The same procedure was done for all the 10 strips of group 1. The same above procedure was followed for all 10 samples of group 2, 3, 4, 5 and 6 groups. The results were compared among the lemongrass oil mouthwash 0.25% and 0.5% alcohol, non-alcohol containing mouth wash and Chlorhexidine alcohol and non-alcohol mouthwash. Statistical analysis was done using paired t test for intragroup analysis and ANOVA for intergroup analysis.

RESULTS AND DISCUSSION

Paired t test: There was a significant difference between the pre and post scores with respect to group 1, 3, 4, 5 and 6. No significant difference was seen with respect to group 2 with respect pre and post scores. ANOVA test: The mean difference in the plaque scores was compared among all the groups. There was no significant difference in the mean difference scores among the study groups. Table 1&2.

Dental plaque is a biofilm adhering to the tooth surface or other hard surfaces in the oral cavity including removable and fixed restoration. It can be readily visualized on teeth after 1 – 2 days with no oral hygiene. A common method of detecting the plaque is by the use of disclosing agent. The various available disclosing agents are erythrosine (PLAKSEE), two tone dye (Alpha Plaque), PLAKLITE, Skinners iodine, Mercurochrome solution (0.5%), Bismark brown (Easlick disclosing solution) and Malachite green.[7,8]

Removal of dental plaque on a regular basis and prevention of its accumulation on teeth is the critical component of regular oral care. Even though the mechanical plaque removal remains the primary method used to maintain oral health; an improved understanding of the infectious nature of the dental disease has revitalized the interest in chemical methods of plaque control.

Mouth washes containing essential oils are used for many years in the prevention and treatment of periodontal disease. Recent studies have
demonstrated that essential oil mouth washes was effective as chlorhexidine mouthwash in inhibiting the plaque regrowth \cite{9,10} as they can penetrate the plaque biofilm, kill the pathogenic microorganisms by disrupting their cell wall and inhibit their enzymatic activity.\cite{11}

Essential oil mouth wash prevent bacterial aggregation, slows their multiplication and extract the bacterial endotoxins.\cite{12} The mechanisms by which essential oils can inhibit microorganisms may be due to their hydrophobicity, due to which they get partitioned into the lipid bilayer of the cell membrane, rendering it more permeable, leading to leakage of vital cell contents.\cite{13} Impairment of bacterial enzyme systems may also be a potential mechanism of action. This suggests that an effective mouthwash must also penetrate the plaque biofilm.

The present study was done to check the anti-plaque efficacy of lemongrass oil mouthwash where the plaque is a biofilm and lemongrass oil mouthwash at both the concentrations showed decrease in the plaque. The anti-biofilm activity can be attributed to the presence of various constituents such as citral, limonene, citronellal, β-myrcene, linalool and geraniol.\cite{14} In the present study Chlorhexidine mouthwash, lemongrass oil mouthwash 0.25% and lemongrass oil mouthwash 0.5% with alcohol showed decrease in plaque biofilm than the mouthwash prepared with alcohol. It has been shown that chlorhexidine binds to salivary mucins on the bacterial cell membrane, and penetrates the plaque biofilm.\cite{11} Lemongrass oil has antibacterial property and also anti-biofilm property which brings about decrease in the bacterial load and inhibits plaque biofilm formation. Based on this above property, lemongrass oil mouthwash can be used as adjunct to mechanical plaque control in the prevention of gingival and periodontal disease.

**CONCLUSION:**

Strength of the study: Aim of the study was met. It was an in-vitro study which depicts the efficacy of mouthwash using the erythrosine dye which has a specific wavelength.

Weakness of the study: As all the polymethy methacrylate strips were kept together in the pooled saliva, the plaque must have disrupted while they were taken for testing rubbing each other. Further studies need to be done in vivo to affirm the results.

The Lemongrass oil 0.5% alcohol and non-alcohol mouthwash can be used as an adjunct to mechanical oral hygiene.

**REFERENCES:**

### TABLES:

#### Table 1: Intra-group analysis

<table>
<thead>
<tr>
<th>Group 1 LGO 0.25% with alcohol</th>
<th>Group 2 LGO 0.25% without alcohol</th>
<th>Group 3 LGO with alcohol 0.5%</th>
<th>Group 4 without alcohol 0.5%</th>
<th>Group 5 Chlorhexidine with alcohol</th>
<th>Group 6 Chlorhexidine without alcohol</th>
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<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<tr>
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<td>.026</td>
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<td>&lt;0.001</td>
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#### Table 2: inter-group analysis for the difference in pre-post scores

<table>
<thead>
<tr>
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<th>3</th>
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<tr>
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<td>.35</td>
<td>.02</td>
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<td>.05</td>
<td>.05</td>
<td>.04</td>
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