



GLOBAL JOURNAL OF MEDICAL RESEARCH

Volume 12 Issue 7 Version 1.0 Year 2012

Type: Double Blind Peer Reviewed International Research Journal

Publisher: Global Journals Inc. (USA)

Online ISSN: 2249-4618 Print ISSN:0975-5888

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GJMR-L Classification : NLMC Code: WB 350, WU 158, WU 113, WU 101.5



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Antiplaque Efficacy of Lemongrass Oil Mouthwash – An in-vitro Study

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I. INTRODUCTION

Removal of dental plaque on a daily routine is one of a major factor in the prevention of caries, gingivitis and periodontitis. Dental plaque accumulation is the pre requisite for the development of gingivitis (Loe H, 1965, p-607). 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 of the disease.

Plaque control can be obtained through the mechanical removal of the biofilm by proper use of tooth brushing technique and flossing. Potential removal of supragingival bacterial plaque by means of tooth tooth brush remains the most widely accepted method of oral disease prevention. The compliance of the patient in conducting routine dental care reduces over a period of time even after education and motivation of the patients. This results in retention of plaque in interproximal surfaces of the teeth.

Chemical control of plaque is considered to be adjunct to mechanical oral hygiene practices, the agents are most commonly used in the form of mouth rinse to prevent and control the plaque formation. Chlorhexidine digluconate is to date is a gold standard, most thoroughly studied and most effective antiplaque and anti-gingivitis agent when addressing oral hygiene (Gjerme P, 1989, p-1602). However several side effects are also associated with its use like staining of teeth and restorations, unpalatable taste with taste alteration 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 one of the important essential oil, extracted from Lemon grass which belongs to the section of Andropogon called Cymbopogon of the family Germinaeae. The botanical genus name Cymbopogon for lemongrass is derived from Greek 'cymbo' 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 has plethora of medicinal uses. It is said to have antibacterial (Pabuseenivasan S 2006, p-39), anti-inflammatory (Carbajal D, 1989, p-1983) antioxidant (Rabbani, S.I, 2005, p-28), antifungal (Taweekaisupapong S, 2012, p-37) antiseptic, astringent, analgesic, antipyretic and carminative property. Because the herb has not been studied extensively, its effectiveness is based mainly on its centuries-old reputation as a folk remedy. Considering the various uses of lemongrass oil an attempt is being made to harness the properties, use of lemongrass oil as a mouth rinse was planned for its antiplaque property. The aim of the present study was to evaluate the efficacy of lemongrass oil mouth-rinse as a chemical plaque control agent in- vitro, by assessing the reduction in the plaque and comparing lemongrass oil mouth wash with chlorhexidine mouth wash, glodent tooth paste and distilled water.

II. MATERIALS AND METHODS

The present work was an in-vitro study in which the materials used were tissue culture plate (6x4 wells), micropipette; set at 10ml, micropipette tips, pooled saliva from the volunteers, lemongrass oil mouthwash 0.5% and 0.25%, chlorhexidine mouthwash, glodent

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toothpaste slurry, erythrosine disclosing agent, ELx 800Ms (ELISA reader).

Tissue culture plate was taken and in each well 10 ml of the pooled saliva was added and was kept in incubator for 72 hours at 37° C, which is equivalent to the temperature of the oral cavity. After 72 hours the saliva present in the wells of the tissue culture plates are removed from the wells of the tissue culture plates which left behind the plaque that was formed at the base and around each well.

1st row of wells (4 wells) was taken as control in which only disclosing agent was added and after 30 seconds it was rinsed with distilled water with the help of micropipette. In the second row of wells lemongrass oil mouthwash (0.5%) was added, kept for 30 seconds and was pipetted out. Later one drop of disclosing agent

was added, kept for 30 seconds after which it was rinsed with the distilled water with the help of micropipette. Likewise in the third row lemongrass oil (0.25%), fourth row distilled water, fifth row glodent toothpaste slurry, and sixth row chlorhexidine 0.2% was added and same procedure was repeated as it was followed in the second row of wells in tissue culture plate. After using all the different agents 10ml of distilled water was added in all the wells and was kept in the ELx 800Ms machine for the analysis. The ELx 800Ms was set at 540nanometer as the absorbency range of erythrosine was 525- 530nanometer. The readings were obtained by the printer connected to the machine. The results were tabulated using the One way analysis of variance (ANOVA) followed by post-hoc Tukey's test.

	N	Mean	SD	p-value	Post-hoc test
1	4	.5715	.10741	0.003	1>2,3 4>2,3
2	4	.3755	.04429		
3	4	.3858	.08213		
4	4	.5635	.06457		
5	4	.4450	.07875		
6	4	.4302	.03642		

Table 1 : Comparative evaluation of lemongrass oil 0.5%, 0.25%, chlorhexidine mouthwash, glodent toothpaste slurry and distilled water.

Sample	Disclosing solution	LGO mouth wash 0.5%	LGO mouth wash 0.25%	Distilled water	Glodent tooth paste slury	Chlorhexidine mouthwash
1	0.557	0.410	0.495	0.526	0.447	0.427
2	0.567	0.379	0.323	0.542	0.481	0.442
3	0.705	0.401	0.403	0.653	0.418	0.412
4	0.592	0.312	0.322	0.508	0.395	0.383
Total	2.421	1.502	1.543	2.229	1.741	1.664
Mean	0.60525	0.3755	0.38575	0.55725	0.43525	0.416

Table 2 : Readings obtained: ELISA Reader (ELx800MS) → 540nm.

III. RESULTS

Multiple comparisons were performed using One way analysis of variance (ANOVA) followed by post-hoc Tukey's test. Overall there was a significant difference in the mean scores between the groups ($p=0.003$). Post hoc analysis showed that group 1 had significantly higher mean than group 2 and 3. Similarly group 4 had significantly higher mean than group 2 and 3. Optical density due to the addition of disclosing agent was more for group 1 and 4 than group 2 and 3 implies that the group 2 and 3 had significantly less amount of plaque than group 1 and 4. There were no significant differences between Group 6 and other materials.

IV. DISCUSSION

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. Plaque is whitish, grayish / yellow and has globular appearance. Plaque is typically observed on the gingival 3rd of the tooth surface (Newman, 2005, p-98). A common method of detecting the plaque is by the use of disclosing agent. They are available in tablet, lozenges or wafers, which contain dye or other colouring agents. 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 (Wilkins EM, 1983, p-405), (Woodal, IR, 199, p-288).

The disclosing solution chosen for the study was erythrosine as it had a single wavelength, which can be easily measured by using the ELISA reader. Erythrosine is a highly coloured molecule that absorbs light near 500nm and emits longer wavelength. The λ max of erythrosine was 525nm, as UV spectrum of erythrosine showed maximum absorbance at 529nm (Ramakrishnan SP, 2007, p-361). In another study by Tinsley D and RG Chadwich (1997) said that the λ max of erythrosine was 530 nm (Tinsley D, 1997, p-67). Based on the above studies the wavelength in the present study was set at 540nm.

The interaction between saliva-coated tooth surfaces and pathogenic bacteria is partly governed by electrostatic and hydrophobic interactions, providing a solid rationale for using chemical agents as part of a plaque-control routine (Rosin M, 2002, p-392). 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 (Rosin M, 2002, p-392), (Riep BG, 1999, p-164) as they can penetrate the plaque biofilm, kill the pathogenic micro-organisms by disrupting their cell wall and inhibit their enzymatic

activity (Ouhayoun JP, 2003, p-10). Essential oil mouth wash prevent bacterial aggregation, slows their multiplication and extract the bacterial endotoxins (Seymour R, 2003, p-10). 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 (Burt S, 2004, p-223), (Juven J, 1994, p-626), (Kim J, 1995, p-2839). Impairment of bacterial enzyme systems may also be a potential mechanism of action (Wendakoon C, 1995, p-280). This suggests that an effective mouthwash must also penetrate the plaque biofilm.

In the present study lemongrass oil has shown be an effective antiplaque agent at both 0.5% and at 0.25% in the mouth wash which was more effective than that of the chlorhexidine. The glodent toothpaste slurry had also reduced the plaque but to a lesser extent than both the lemongrass oil 0.25% and 0.5% and chlorhexidine mouth wash. (Table 1 and 2) The present study can be related to the study done by S. Taweechaisupapong et al 2012, where they stated that exposure of *Candida* cells to subcidal concentration of lemongrass oil can reduce the adherence ability of the cells in inhibitory effect on biofilm formation (Taweechaisupapong S, 2012, p-37). Since adherence represents a major step in biofilm formation and lemongrass oil might be used to prevent *Candida* biofilm associated infection (Taweechaisupapong S, 2012, p-37). The present study was done to check the anti- plaque efficacy of lemongrass oil mouthwash where the plaque is a biofilm and lemongrass oil mouth wash 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 (Raubert Cd, 2005, p-597), (Schanebortoryerg, 2002, p-1345), (Tognolini, 2006, p-1419). In the present study Chlorhexidine mouth wash also showed decrease in plaque biofilm. It has been shown that chlorhexidine binds to salivary mucins on the bacterial cell membrane, and penetrates the plaque biofilm (Ouhayoun JP, 2003, p-10). 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.

V. CONCLUSION

Lemongrass oil mouthwash at both 0.25% and 0.5% was effective in reduction of the plaque. Based on this property, lemongrass oil mouthwash can be used

as adjunct to mechanical plaque control in the prevention of gingival and periodontal disease.

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