Original Research

# Myrrh Oil Reduces Gingival Inflammation and Inhibits Gram Negative Dental Plaque Bacteria at Early Stages – A Randomized Control Trial

# Bhagyashree Lenka<sup>1</sup>, Rinkee Mohanty<sup>2</sup>, Anurag Satpathy<sup>3</sup>

<sup>1</sup>Post Graduate Student, <sup>2</sup>Professor and Head, <sup>3</sup>Professor, Department of Periodontics and Oral Implantology, Institute of Dental Sciences, Siksha 'O'Anusandhan (Deemed to be University), Bhubaneswar, Odisha

#### **Abstract**

Aim: To assess the anti-plaque, anti-inflammatory and antimicrobial efficacy of Myrrh oil as an adjunct to scaling and root planing in the treatment of gingival inflammation. Materials and Methods: Subjects with moderate to severe gingivitis were recruited for this parallel arm, double blind randomized controlled trial. All subjects underwent SRP at baseline and were randomly divided into Group A (placebo control) and Group B (Myrrh oil). Plaque index (PI), gingival index (GI) and microbiological analysis was carried out at baseline, 48 hrs and 1 week interval **Results**: Thirty subjects completed the study. Myrrh oil showed anti-inflammatory and antibacterial efficacy. There were no statistically significant differences between the groups with respect to plaque score at baseline (p=0.25), after 48 hours (p=0.16) and after 1 week (p=0.37). There was a significantly lower gingival inflammation (p=0.02) recorded in the Myrrh oil group after 48 hrs. A greater reduction in inflammation from baseline at 48hrs was observed, although there was no statistically significant difference in gingival inflammation between the groups after 1 week (p=0.39). A significantly greater number of fields were observed with score 1 and lesser number of fields with 2 for gram +ve (p<0.001) and gram -ve (p=0.002) bacteria in Myrrh Oil group indicating overall lesser gram +ve and Gram -ve bacterial count in comparison to commercially available Myrrh oil. No adverse effects were reported by any subject. Conclusion: Myrrh oil when used as an adjunct with scaling and root planning significantly reduced the gingival inflammation in 48 hrs and gram-ve bacteria after 1 week.

Key words: myrrh oil; essential oil; anti-inflammatory; antibacterial; gram staining

# Introduction

Gingival and periodontal diseases are caused by bacteria and bacterial products in the dental plaque. <sup>[1,2]</sup> Dental plaque exists as a biofilm on the surfaces of tooth and mucosal surfaces. <sup>[3,4]</sup> In addition to mechanical plaque removal by scaling and root planing (SRP), chemical plaque control is often advocated as an adjunct to reduce plaque formation and plaque-associated gingivitis. <sup>[5]</sup>

# Corresponding Author: Prof. Anurag Satpathy,

Department of Periodontics, Institute of Dental Sciences, Siksha 'O' Anusandhan University, Khandagiri Square, Bhubaneswar – 751030, Odisha, India. E mail: drasatpathy@gmail.com Although chlorhexidine gluconate has been considered as the gold standard among anti-plaque agents, altered taste sensations, staining of teeth, and development of resistant microorganisms have been observed as its undesirable side effects when used on a long term basis. <sup>[6]</sup> Similarly, several chemical astringents have been used to reduce gingival inflammation and gingival bleeding. It necessitates the development of alternate anti-plaque and anti-inflammatory agents for prevention of dental plaque and gingivitis. In this endeavour, several plant and animal-based natural products have found use in the treatment of oral ailments<sup>[7]</sup>; these include Triphala (Emblica officinalis<sup>[8]</sup>), Turmeric (Curcuma longa) <sup>[9]</sup>, Basil (Ocimum tenuiflorum)<sup>[10]</sup>, Neem (Azadirachta indica<sup>[11]</sup>), Miswak (Salvadora persica)<sup>[12]</sup> and Honey.

[13,14]

Myrrh essential oil is an extract of Myrrh gum which is harvested from the species *Commiphora myrrha*. It is a plant extract with medicinal properties with a history that dates back to the birth of Jesus in 6<sup>th</sup> BC where the three kings who travelled to visit Jesus after his birth, bearing gifts of gold, Frankincense, and myrrh. Myrrh is said to have therapeutic properties since ancient times. Myrrh is has a stimulating action for blood circulation, soothes pain and provides relief from swelling. It helps in the regeneration of skin and accelerates the healing of skin ailments.

Historically, Myrrh tincture has also been used as a mouthrinse in the treatment of oral ulcers and gingival inflammation. [15] It has also been used topically in the treatment of inflammation of the oro-pharynx. [16] There is no recent reported literature or product where Myrrh oil has been clinically used in the treatment of periodontal disease. Therefore, the present study was carried out to assess the anti-plaque, anti-inflammatory and antimicrobial efficacy of Myrrh oil as an adjunct to scaling and root planing in the treatment of gingival inflammation.

### **Materials and Methods**

Study Population and Study design

Patients reporting to the Outpatient Department of Periodontics and Oral Implantology were screened for recruitment in the study. This study was a parallel arm, double-blind, randomized controlled clinical trial. The institutional review board approved the study and written informed consent was taken from the patients before inclusion in the study. Patients fulfilling the following eligibility criteria were selected:

#### Inclusion criteria

- · Aged 18–35 years
- · No history of any systemic diseases
- · Possessing a minimum of twenty permanent natural teeth with no visible signs of untreated caries
- · Moderate to Severe gingivitis ( Gingival Index score  $\geq 2$ )<sup>[17]</sup>

#### **Exclusion criteria**

- Patients with a history of use of antibiotics within the last three months
  - · Pregnant women and lactating mothers
  - Medically compromised patients
  - · Smoking or smokeless tobacco users
  - · Patients with removable or fixed appliances
- · Participants with a known history of allergy to any chemical or herbal products

#### Randomization, blinding and allocation

Randomization of test and control products was done by a simple coin test method by a blinded examiner (RM) to Group A, and Group B. Group A subjects received Glycerol (Kazima Perfumers, New Delhi, India) as a placebo control while the subjects in Group B received Myrrh oil (Kazima Perfumers, New Delhi, India) respectively.

#### Procedure

Microbial dental plaque and gingival inflammation were assessed at baseline using plaque index (PI) [18] and gingival index (GI). [17] Supragingival plaque samples were also collected at baseline from the buccal surface of the upper first permanent molar using a curette. Each participant underwent scaling and polishing following the assessment of baseline plaque and gingival inflammation status and collection of plaque samples. Subsequently, the participants were supplied with the allocated placebo/test product dispensed in amber-coloured droppers to mask the colour hue. They were instructed to apply the two drops of the dispensed product twice daily with their finger on the gums for 1 minute and rinse with water after tooth brushing. The technique of application and tooth brushing (Modified Bass [19]) was demonstrated by a trained investigator to all participants. The post-intervention assessment and sample collection was done by the same investigators after 48 hours and after 1 week.

## Microbiological Analysis

For microbial analysis, gram staining was used. [20] Collected microbial plaque samples were spread on

sterile microscopic slides and were stained with Gram's stain. Slides were then examined at 100X magnification with a light microscope. Quantitative assessment of the bacteria was done by visualizing five random non-overlapping fields. Scoring was done as per the number of bacteria visible in each field. Fewer than five visible bacteria were assigned a score of 1, 5-10 visible bacteria were scored as 2, 10-20 visible bacteria were scored as three and more than 20 visible bacteria were scored as 4. To compute the number of bacteria, the number of observed fields was multiplied with the score. All clinical recordings and microbial sampling was done by a single investigator (BL) who was blinded to the allocation.

# **Statistical Analysis**

A sample size analysis was estimated using G\*Power software version 3.0.10 (Universitat Kiel, Germany) with 5% significance level, 80 % power and effect size of 0.5 estimated the total sample size to be 27. Since there were two groups and it was a parallel arm study 20 subjects were enrolled in each group taking in account the possible 10% drop-outs during follow up. Data was analyzed using Statistical Package for Social Sciences Version 20.0 (SPSS Inc, Chicago Illinois, USA). Collected data were tested for significant differences within the groups and between the groups with the Student's paired and unpaired t-tests. A value of  $P \leq 0.05$  was considered statistically significant for all analyses.

#### **Results**

A total of 30 (males =14; females 16) subjects aged  $24.19 \pm 2.63$  years completed the study successfully. 5 subjects dropped out in each group owing to noncompliance (Fig 1). None of the subjects reported any adverse effects or any minor side effects due to any of the products during the study period. Table 1 presents the comparison of clinical parameters between Group A and Group B at baseline, 48 hours and after 1 week. There were no statistically significant differences between the groups with respect to plaque score (PI) at baseline

(p=0.25), after 48 hours (p=0.16) and after one week (p=0.37). Although there was a significant decrease in the plaque scores from baseline to that at 48 hrs, it increased significantly after an initial decrease (Figure 3) as observed after one week. Also, there were no statistically significant differences between the groups with respect to gingival inflammation (GI) at baseline (p=0.51). However, there was a significantly lower gingival inflammation (p=0.02) recorded in the Myrrh oil group after 48 hrs.

Further, a greater reduction in inflammation was seen from baseline to that at 48hrs (Figure 4), although there was no statistically significant difference in gingival inflammation between the groups after1 week (p=0.39). The intra-group comparisons (Table 2) revealed that there was a significant reduction in the plaque score and gingival inflammation at 48hrs and 1 week from baseline in both groups. However, there was no statistically significant further reduction in plaque score (p=0.107) and gingival inflammation (p=0.112) from 48hrs to 1 week.

Comparison of microbial count between Group A and Group B at baseline and 48 hours (Table 3) and one week (Table 4) reveals that there was no statistically significant difference in the number of gram +ve and Gram –ve bacteria between the groups at baseline. However, after 48 hrs, a significantly greater number of fields were observed with score one and lesser number of fields with score 2 for Gram +ve (p < 0.001) and Gram –ve (p=0.002) bacteria in Myrrh Oil group indicating overall lesser gram +ve and Gram –ve bacterial count.

After one week, there was no significant difference between the groups in the Gram +ve bacterial count but showed a significant reduction in the microbial count of Myrrh oil group in the fields with score 4 (more than 20 visible bacteria) (p=0.042) and overall count (p=0.018) indicating a gradual decrease in the total number of a gram-negative microorganism after one week in the Myrrh oil group.

Table 1. Comparison of plaque score and gingival inflammation scores between Group A (Placebo) and Group B (Test) at baseline, 48hrs and after 1 week.

	Cwoun	N	Mean	SD	95% CI		p value*
	Group				Lower	Upper	p value
Plaq	ue Score						
Baseline	Group A	15	3.27	0.40	-0.13	0.47	0.25
Daseille	Group B	15	3.10	0.39			0.23
after 48 Hrs	Group A	15	1.86	0.15	0.47	0.08	0.16
aner 48 mis	Group B	15	2.05	0.49	-0.47		0.10
A C+ 1 1-	Group A	15	2.41	0.25	-0.09	0.24	0.37
After 1 week	Group B	15	2.34	0.18			
Gingival	Inflammation						
D1:	Group A	15	1.86	0.31	0.12	0.16	0.51
Baseline	Group B	15	1.94	0.34	-0.12		
0 40 11	Group A	15	1.48	0.36	0.04	0.56	0.02
after 48 Hrs	Group B	15	1.18	0.33			0.02
A C 1 1	Group A	15	1.03	0.44	0.54	-0.19	0.20
After 1 week	Group B	15	0.93	0.33	-0.71		0.39

N= Number of subjects; \*Unpaired t test; p < 0.05; SD= Standard Deviation; CI= Confidence Interval

Table 2. Intra-group Comparisons of Plaque Score and Gingival Inflammation for Group A and Group B

		N.T	Maan	CD	CEM	95% CI		D.V. 1. //	
		N Mean SD	SEM	Lower	Upper	P Value#			
	Plaque Score								
	At Baseline	15	3.27	0.40	0.10	3.05	3.49		
	After 48 Hrs	15	1.86	0.15	0.04	1.77	1.95	< 0.001	
	After 1 Week	15	2.41	0.25	0.07	2.27	2.55		
Group A	F=92.28; df = 42;2	Baseline-48 hrs(<0.001)*; Baseline-1 week (<0.001)*; 48 hrs-1 week (<0.001)*							
Grou	Gingival Inflammation								
	At Baseline	15	1.86	0.31	0.08	1.69	2.03		
	After 48 Hrs	15	1.48	0.36	0.09	1.28	1.68	<0.001	
	After 1 Week	15	1.03	0.44	0.11	0.79	1.27		
	F=18.89; $df=42$ ;2	Baseline-48 hrs(<0.001)*; Baseline-1 week (=0.021)*; 48 hrs-1 week (=0.005)*						(=0.005)*	

Cont... Table 2. Intra-group Comparisons of Plaque Score and Gingival Inflammation for Group A and Group B

	Plaque Score								
	At Baseline	15	3.10	0.39	0.10	2.89	3.32		
	After 48 Hrs	15	2.05	0.49	0.13	1.78	2.33	<0.001	
В	After 1 Week	15	2.34	0.18	0.05	2.24	2.44		
Group F	F=92.28; df = 42;2	Baseline-48 hrs(<0.001)*; Baseline-1 week (<0.001)*; 48 hrs-1 week (=0.107)						=0.107)*	
Gre	Gingival Inflammation								
	At Baseline	15	1.18	0.33	0.09	1.00	1.36		
	After 48 Hrs	15	0.93	0.33	0.08	0.75	1.11	<0.001	
	After 1 Week	15	1.35	0.54	0.08	1.19	1.51		
	F=18.89; df = 42;2	Baseline-48 hrs(<0.001)*; Baseline-1 week (<0.001)*; 48 hrs-1 week (=0.112)*							

N= Number of subjects; SD= Standard Deviation; SEM= Standard Error of Mean; # One-Way ANOVA Test; \* Tukey's Test for multiple comparison; p<0.05; SD= Standard Deviation; CI= Confidence Interval

Table 3. Comparison of bacterial count between Group A (Placebo) and Group B (Test) at Baseline and after 48 hrs.

			At Baseline		After 48 Hrs			
Parameter	Group	N	Mean	SD	p value*	Mean	SD	p value
Gram+ve ( <5 )	Group A	15	0.53	0.64	0.702	1.73	1.33	< 0.001
	Group B	15	0.6	0.74	0.793	3.27	0.59	<0.001
C (5.10)	Group A	15	1.73	1.67	0.432	4.67	2.69	< 0.001
Gram +ve (5-10)	Group B	15	2.27	1.98	0.432	1.73	1.03	<0.001
Gram +ve (10 - 20)	Group A	15	5.6	2.97	0.866	1.6	2.5	0.702
Gram +ve (10 - 20)	Group B	15	5.4	3.44	0.800	1.8	1.52	0.793
Cross 1222 (> 20)	Group A	15	7.2	4.59	0.472	1.6	2.95	0.556
Gram +ve (>20)	Group B	15	5.87	5.42	0.473	1.07	1.83	
Cross Lyza Tatal	Group A	15	15.07	2.91	0.407	9.6	2.97	0.046
Gram +ve Total	Group B	15	14.13	3.16		7.87	1.25	
Crome via ( < 5 )	Group A	15	0.67	0.72	0.63	1.53	1.25	0.01
Gram –ve ( <5 )	Group B	15	0.8	0.77		2.67	0.98	
Cross vs (5.10)	Group A	15	2.13	1.6	0.201	4.67	3.18	0.002
Gram -ve (5-10)	Group B	15	3.07	2.25	0.201	1.47	1.92	
C (10, 20)	Group A	15	4	2.45	0.457	1.4	1.92	0.148
Gram -ve (10-20)	Group B	15	3.2	3.3	0.457	2.8	3.1	
Gram -ve (>20)	Group A	15	7.73	6.32	0.564	2.93	2.81	0.765
	Group B	15	6.4	6.2	0.564	2.67	1.95	0.765
C T-t 1	Group A	15	14.53	3.11	0.386	10.53	1.92	0.125
Gram -ve Total	Group B	15	13.47	3.5		9.6	1.35	0.135

N= Number of subjects; \*Unpaired t test; p < 0.05; SD= Standard Deviation; CI= Confidence Interval

Table 4. Comparison of bacterial count between Group A (Placebo) and Group B (Test) 1 Week

Parameter	Cmann	N	Mean	Std. Deviation	95%	1 4	
i ai ametei	Group				Lower	Upper	p value*
	Group A	15	0.73	0.88	-1.41	-0.19	0.012
Gram+ve( <5)	Group B	15	1.53	0.74			
G (5.10)	Group A	15	2.40	1.88	-0.94	2.01	0.466
Gram +ve (5-10)	Group B	15	1.87	2.07			
C (10, 20)	Group A	15	2.80	2.40	-1.63	2.03	0.825
Gram +ve (10 - 20)	Group B	15	2.60	2.50			
C ( 20)	Group A	15	8.53	3.96	-1.93	5.66	0.322
Gram +ve (>20)	Group B	15	6.67	5.98			
	Group A	15	14.47	3.02	-0.65	4.25	0.144
Gram +ve Total	Group B	15	12.67	3.52			
G (15)	Group A	15	0.93	1.03	-1.25	0.31	0.231
Gram -ve( <5 )	Group B	15	1.40	1.06			
(5.10)	Group A	15	1.60	1.72	-2.64	0.24	0.099
Gram -ve (5-10)	Group B	15	2.80	2.11			
G (10.20)	Group A	15	5.20	3.67	-0.99	3.79	0.241
Gram -ve (10-20)	Group B	15	3.80	2.65			
G (20)	Group A	15	6.13	3.34	0.09	4.71	0.042
Gram -ve (>20)	Group B	15	3.73	2.81			
	Group A	15	13.87	2.59	0.39	3.88	0.018
Gram -ve Total	Group B	15	11.73	2.05			

N= Number of subjects; \*Unpaired t test; p < 0.05; SD= Standard Deviation; CI= Confidence Interval

Figure 1. CONSORT Workflow Diagram

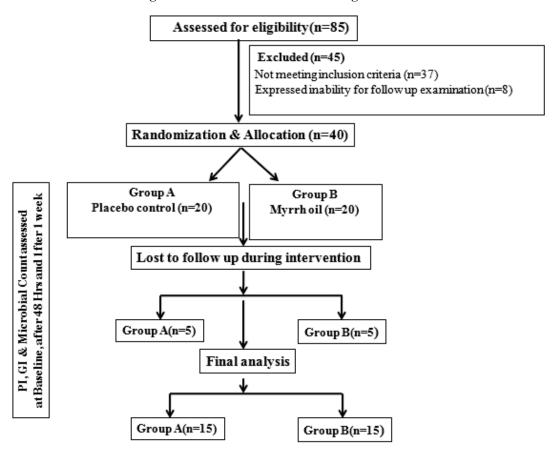
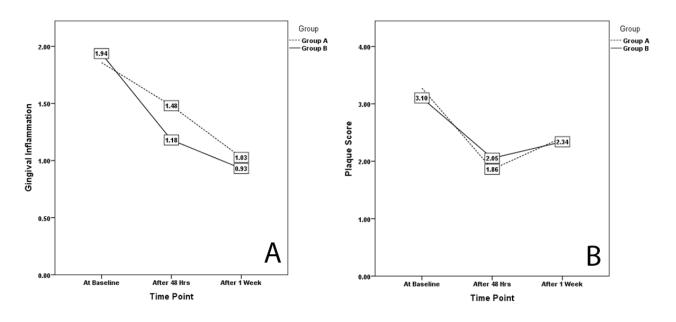


Figure 2. Gingival Inflammation Score (A) and Plaque score (B) of Group A (Placebo) and Group B (Test) at baseline, 48hrs and after 1 week.



#### **Discussion**

The microbial dental plaque remains to be the primary agent in causing inflammatory periodontal disease. [21,22] Although mechanical plaque inhibition continues to be the mainstay in the prevention of periodontal disease, several plaque inhibiting products have been regularly used as an adjunct.

The present study assessed the anti-plaque, anti-inflammatory and antimicrobial efficacy of Myrrh oil for seven days. While there was no significant difference in their anti-plaque efficacy over a week, there was a significant reduction in the gingival inflammation after one week in Myrrh oil group. There, however, was an increase in the plaque level after 1 week after it initially decreased after 48 hours.

We found a significant antibacterial effect of Myrrh oil, a plant extract leading to reduction of bacterial count (Gram -ve microorganisms) 7 days after the intervention compared to baseline levels. This finding was in accordance with a study by Sukhabogi et al. [23] who compared three different herbal products with a chlorhexidine gel and found to be effective in reducing plaque and gingival scores. Al-Mobeeriek et al. [24] in an animal study reported that myrrh suspension promotes healing and repair of damaged tissue in comparison to Chlorhexidine and Tetracycline. Similarly, Gupta et al. [25] in their in vitro study also demonstrated the antimicrobial efficacy of aqueous and ethanolic extracts of Triphala on primary plaque colonizers. Chandrashekar et al. [26] in their in vitro studies also demonstrated the antibacterial efficacy of eucalyptus plant extracts on plaque microorganisms. Plant-based medicines are adjunct to mechanical plaque control methods and are being established as an alternative to chlorhexidine. Absence of adverse effects by plant products when used over a long period of indicates their safety when used as an anti-plaque, anti-inflammatory or antimicrobial oral formulation. Prakash and Shelke et al. [8] have shown that Triphala is efficacious in reducing microbial plaque and controlling gingivitis. Herbal dentifrice formulations containing Cinnamomum camphora and menthol have been found to reduce plaque in patients with gingival inflammation. [27]

Myrrh (*Commiphora myrrha*) oil contains a-pinene, cadinene, limonene, cumin aldehyde, eugenol,

m-cresol, heerabolene, acetic acid, formic acid and other sesquiterpenes and acids. The antibacterial and anti-gingivitis effect is mainly attributed to these ingredients. Myrrh has been reported to be highly effective in the treatment of inflammatory diseases such as Rheumatoid arthritis. In an in-vivo study by Su et al. To revealed a reduction in elevated expression levels of TNF $\alpha$ , PGE2, IL-2 and Nitric Oxide in serum after treatment with Myrrh and Frankincense. The previous study by Ljaljević Grbić et al. The previous study by Ljaljević Grbić et al. The previous demonstrated the susceptibility of Gram-positive and Gram-negative bacteria Myrrh oil (MIC 2–5  $\mu$ L/mL to 100–1000  $\mu$ L/mL).

#### **Conclusions**

This was first of its kind study to assess the antiplaque, anti-inflammatory and the antibacterial inflammation of Myrrh oil on its topical intraoral application. Although gram staining tells us about the presence of gram-positive and the Gram negative bacteria, it is a weak indication of antibacterial activity as compared to culture studies and polymerized chain reaction. Also, in such kind of studies, Hawthorne effect may also act as a bias as it influences the performance of the participating subjects. Within the limitations of the study, it can be concluded that Myrrh oil is an efficacious anti-inflammatory and antibacterial oral formulation when used as an adjunct to scaling and root planning in the treatment of gingivitis.

**Ethical Permission**: Approved

Conflicts of Interests: None

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