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Effectiveness of Myristica fragrans mouth rinse in Children: A clinical trial

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Abstract

Aim: The aim of the study was to compare the efficacy of Myristica fragrans (Nutmeg) mouth rinse with 0.2% chlorexidine mouth rinse.

Materials and methods: A double-blinded randomized clinical trial was conducted on 7-12 year old children for duration of 30 days. Streptococcus mutans count (CFU-colony forming units), Plaque index (PI) and Gingival index (GI) was recorded at base-line (pre-rinse), 30 minutes post rinse and 15th and 30th day. The data regarding patient compliance was also collected through a validated questionnaire.

Results: Myristica fragrans mouth rinse was effective in reducing S mutans count and was comparable to 0.2% chlorhexidine in bringing about improvement of Plaque index and gingival index scores. Nutmeg mouthwash was accepted by 93.3% of the study participants and only 80% of them were willing to use.

Conclusion: Nutmeg mouth rinse showed significant reduction in S mutans count and was comparable with

0.2% chlorhexidine in S mutans count, Gingival index score and Plaque scores. Nutmeg mouthwash was well accepted by children. Hence Myristica fragrans mouthwash can be used as suitable alternative to chlorhexidine 0.2% mouthwash in children.

Keywords: Mouth rinse; Myristica fragrans; Plaque index; S mutans

Introduction

Dental diseases are recognized as major public health problem throughout the world. Numerous epidemiological studies showed that diseases such as tooth decay and diseases of the periodontium are among the most common afflictions of mankind. Dental plaque plays a major role in the etiology of dental caries and periodontal disease and also dental caries is the most common disease affecting children worldwide. The mainstay of preventing these dental diseases is the control of plaque.[1,2]

Most of the chemical products contain an antiseptic that plays an important role in controlling plaque accumulation. The vehicles for delivery of chemical agents with anti- plaque action are toothpaste, mouthwashes, spray, irrigators, chewing gums, and varnishes.[3,4] The most accepted method of delivering the anti-microbial agents after toothpaste is mouthwashes.[5,6] Mouthwashes are an antiseptic solution which is used to reduce the microbial load in the oral cavity. Mouth rinses have the ability to deliver the therapeutic effect all over the tooth surface including interproximal areas in which even toothpastes are not much effective.[7]The benchmark control in the removal of plaque is chlorhexidine which is considered as Gold Standard. But it cannot be used for a long duration because it has many side-effects like altered taste sensation and staining of tongue. Chemical plaque control agents are used as an adjuvant since they have the ability to inhibit growth and metabolism as well as colonization of bacteria; however, all are associated with various side effects.[8,9,10] Hence there is shift of attention toward herbal drugs. Plants and plant extracts demonstrate effects that are immune enhancing, anti-bacteria, anti-inflammatory, anti-cancer, etc. One such nature's gift is Myristica fragrans.

Myristica fragrans (Nutmeg), indigenous of Moluccas and Banda Island produces two spices- Nutmeg and Mace. It is now cultivated in tropical regions, especially in Indonesia, Grenada in the West Indies Sri Lanka and India (Purseglove,1968; Bown, 1995).[12] It is used as a spice in various dishes, as components of tea and soft drinks or mixed in milk and alcohol. The most important part of the plant in terms of its pharmacological activity and also in commerce is of course the dried kernel (seed), the nutmeg. In traditional medicine nutmeg is sometimes used as a stomachic, stimulant, carminative as well as for intestinal catarrh and colic, to stimulate appetite, to control flatulence and it has a reputation as an emmenagogue and abortifacient (Nadkarni, 1988).[13] Myristica fragrans is widely used as a flavoring agent, a hair dye and a folk medicine. It also possesses anti-papillomagenic, anti-carcinogenic (Hussain & Rao, 1991),[14] anti-inflammatory activities (Ozaki et al., 1989)[15] antioxidant effect, anti-diabetic, hepatoprotective and excellent antibacterial properties.

M fragrans (nutmeg and mace) is known to exhibit strong antimicrobial activity against animal and plant pathogens, food poisoning and spoilage bacteria including Bacillus subtilis, Escherichia coli, Saccharomyces cerevisiae, multi-drug resistant Salmonella typhiand Helicobacter pylori (Orabiet al.,1991; De et al., 1999; Dorman & Deans, 2000; Rani &Khullar, 2004; Mahadyet al., 2005; O'Mahonyet al., 2005).[11,16,17] M. fragrans extracts have shown to be very effective against oral microorganisms like S.mutans.[18,19] It selectively suppressed Porphyrominus gingivalis growth and is a potent natural anti-biofilm agent

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against oral primary colonizers Streptococcus sangius and Actinomyces viscosus (Yanti et al 2008).[19] Thus in our study we compared and evaluated the effect of Myristica fragrans mouth rinse with 0.2% Chlorhexidine, the gold standard on Plaque score, Gingival index and Streptococcus mutans counts.

Materials and methods

A double-blind, randomized, controlled study design was framed for conducting this study. Ethical approval was taken from the university ethical committee for the study (IEC -Dr B R Ambedkar Medical College and Hospital EC-648/2019)). Prior written permission and informed consent were obtained from the parents, head of the Institution and assent from the children.

Based on published literature, the sample size was estimated using G Power Software v.3.1.9.2. Considering the effect size to be measured (f) at 40%, power of the study at 80% and the margin of the error at 5%, the total sample size needed is 45. The samples were divided into 3 groups with 15 samples in each group. The samples were selected based on inclusion and exclusion criteria.

Inclusion criteria: [20]

- Children between the age groups of 7 -12 years children with DMFT/dft 0-6 [21]
- Who are not taking any orthodontic treatment
- Who have given their assent and whose parents had given written informed consent for study [22] Exclusion criteria: [20,21,22]
- Children with mild to severe gingival inflammation
- Who requires special health care and presence of any other systemic medical conditions Preparation of the Myristica fragrans Mouth rinse: [23] Nutmeg authenticated for the study were pulverized (50g) and soaked in 2000ml of distilled water and heated at 100^0C for 30 minutes using a hot plate, filtered using Whatman No 1 filter paper, concentrated in rotatory evaporator, dried in a hot air oven (SANFA DHG - 9202, Gulfex Medical and Scientific England) at 45^0C and stored at 4^0C in refrigerator until further use.

Clinical Procedure: A total of 78 students of the Orphanage (the study was conducted in a home for underprivileged and orphan children in East Bangalore, India) were examined, out of which 45 participants were included in the study. Oral hygiene instructions were given to all the participants. Modified bass technique was demonstrated, and each participant was provided toothbrush and toothpaste on the first visit for use. Baseline plaque index (PI), Gingival index (GI) were recorded and unstimulated saliva was collected for S mutans count.

All 45 participants were categorized as Group 1, Group 2, or Group 3 based on computerized randomization. The label for each mouth rinse group as 1, 2, or 3 was done by co-investigator. List of blinded groups was sealed in an envelope which had been opened only after completion of the study. Based on the grouping, the person who has labeled the groups dispensed the material accordingly. Participants and principal investigator did not know which was the placebo group, positive control group, or experimental group. Group 1 –positive control group: Conventional 0.2% chlorhexidine (HAA, Cadila SMNB/09/41), Group 2 – Experimental group: Myristica fragrans mouth wash, Group 3 – Placebo group-Saline group. All the participants were instructed regarding the use of mouth rinse and were asked to perform the same. After 30 mins, again unstimulated saliva was collected in a sterile disposable container. All children were asked to continue mouth rinsing daily twice for 30 days and unstimulated saliva sample was collected; plaque and gingival index scores were recorded on day 15 and day 30.

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Each saliva sample after collection was analyzed for S mutans count. The Mitis salivarius bacitracin agar (MSBA, Hi Media) media was used in this study for culturing salivary S mutans. The plates were then incubated at 37ºC for 48 h under 5%–10% CO2. To avoid bias, all plates were processed and examined by the principal investigator. The colony count of each plate was recorded, and the mean colony forming units (CFU/ml) were determined. The data regarding patient compliance was also obtained by all the study participants.

The data collected was tabulated and subjected to statistical analyses using SPSS statistical software package, version 22.0. Descriptive analysis of all the explanatory and outcome parameters was performed using frequency and proportions for categorical variables, and using mean standard deviation (SD) and percentages for continuous variables. Multiple comparison of mean, mean difference of CFU, PI and GI between the study groups was performed by Kruskalwallis test and Mann Whitney Post hoc analysis. Mean and Mean difference of CFU, PI and GI at different time intervals in each study group using Friedman's test and Wilcoxon signed Rank Post hoc test. The level of significance was set at $P < 0.05$.

Results

The results of our study showed comparison of S mutans counts scores between different study groups statistically significant difference at various time intervals among the groups except at baseline [pre-rinse] (Table 1, 2 and 3) (Graph 1).

Comparison of Plaque index scores and Gingival index scores between the groups showed no difference at baseline and significant difference between the saline group with other tested groups and also no significant difference between Chlorhexidine and Nutmeg group at 15 days and 30 days. (Table 4, 5, 6 and 7) (Graph 2 and 3).

Within the each tested group comparison of S mutans counts, Gingival index counts and Plaque index scores showed significant difference at different time intervals, baseline, 30 minutes, 15 days and 30 days (Tables 8, 9, 10, 11 and 12) except for S mutans counts and Plaque scores in saline groups between 15 days and 30 days. (Table 8 and 10)

In the present study none of the participants complained of stained teeth or altered taste sensations throughout the study period. About 93.33% of our participants liked Nutmeg mouth rinse and 80% of them were willing to continue the use of mouth rinse as compared to 46.6% liking and 33.33% willing to continue in saline with 20% in Chlorhexidine group liking the taste and willing to continue.

Discussion

Dental caries and periodontal disease are the two most important oral diseases affecting oral health of mankind. Both these diseases are microbial in nature and the surface of the oral cavity is constantly colonized by microorganisms. One milliliter of whole saliva may contain more than 200 million organisms representing more than 250 different species.[24,25] These diseases occur by accumulation of microorganisms in the form of an adherent plaque. Dental caries is the single most common disease affecting the childhood. Children with high DMFT/dft have increased Streptococcus mutans (S mutans) count. Variety of antiplaque agents has been tested for their ability to control S mutans. Streptococcus constitutes an essential part of the micro flora which constantly colonizes the mucous membrane and the teeth. The streptococci in the oral cavity comprise Streptococcus sanguis, Streptococcus mitis, Streptococcus salivarius, Streptococcus intermedius, and other streptococci of which mutans streptococci, especially Streptococcus mutans and Streptococcus sobrinus, are maximum.[26,27]

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Therefore, decreasing the concentration of S mutans in the oral cavity would have a great benefit with respect to decreasing the incidence of dental diseases.

The effect of mechanical oral hygiene techniques on the salivary levels of microorganisms, especially S mutans, is of great interest to dentists focused on preventive care. Tooth brushing with fluoridated toothpaste is considered to be the bed-rock of caries prevention. However, tooth brushing alone is effective in reducing bacterial counts in the mouth, but not dramatically.[28,29] Again, the observations of increasing prevalence of dental fluorosis, suggesting adverse health effects from fluorides, justify a broadening of the current caries prevention strategies that presently rely heavily on fluoride products, to include the incorporation of antimicrobial or other chemotherapeutic agents in dental practice.[30,31] Mouth rinsing as an adjunct to mechanical cleansing became popular with the upper classes in the Roman period, with Pliny recommending salt water as the mouth rinse.[28,32]

Many non- chemical herbal agents have been tried in recent years to provide achievable benefits without or minimal adverse effects. One of the gifts from Mother Nature is Myristica fragrans which has many beneficial effects with excellent antimicrobial properties including microorganisms of oral cavity.[13-20]

In this study we included children aged between 7-12 years in mixed dentition period. Since the intervention in the present study is a mouth rinse, younger children might face difficulties to use it.[33]

As the present study was conducted in residential institution (RTC Orphanage) for Girls, Male subjects could not be selected. However, epidemiological studies in caries prevalence have not shown any significant difference in the caries susceptibility of boys and girls at an average age.[34]

As our study was conducted in an Institution, all the participants consumed the same diet during the period of investigation. As diet (an important factor in dental caries) was uniform among all the groups in our study, the different experimental procedures were possibly given the best chance of demonstrating their efficacy against salivary S mutans and other beneficial effects.

Unstimulated saliva was used for the microbial analysis in the present study as it has been reported by Rupesh et al that the unstimulated saliva represents the basal salivary flow rate.[24,28] Sterile disposable containers were used for collection of unstimulated saliva for microbial analysis in this study. In contrast to most other bacteria, mutans streptococci can grow in an environment with a high sucrose concentration and are resistant to a particular antibiotic, bacitracin, the most commonly used selective medium that is MSBA (Mitis Salivarius Bacitracin Agar). Hence, in the present study, selective media MSBA was used for incubation of salivary S mutans.[34] the technique which was adopted in this study for agar plating and colony counting was similar to that suggested by Wan et al.[35]

Intergroup comparisons were done with Kruskalwallis test and Mann Whitney Post-hoc test. Intergroup comparisons revealed that there was no statistically significant difference in S mutans counts, Plaque index scores and gingival index scores between the groups at baseline (Pre-rinse) implying similar distribution of participants among the groups. (Table 1, 3 and 5)

Intergroup comparison of S mutans count showed statistically significant difference between the groups at 30 minutes post-rinse, 15days and 30 days, with greater reduction in counts with Chlorhexidine followed by Nutmeg and lastly saline group. These findings are similar to a study done by Lakade et al, where 0.2% chlorhexidine showed a greater reduction of S mutans count than

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combination mouth rinse[37]. Similarly, Sharma et al. found that 0.2% chlorhexidine was most effective than combination mouthwash containing 0.03% triclosan and 0.05% sodium fluoride[38]. Shah et al showed that 0.2% chlorhexidine and Oratreat herbal mouthwashes have shown statistically highly significant difference in their efficacy in reducing salivary S mutans when compared with distilled water.[24]

Intergroup comparisons of plaque scores and gingival scores showed statistically significant scores with respect to Chlorhexidine and Nutmeg when compared to saline and no statistically significant difference between Chlorohexidine and Nutmeg (Table 4, 5) at day 30. This finding is similar to study done by Sirjana et al where the herbal mouth rinse showed similar results when compared to Chlorohexidine 0.2% on visually impaired students.[39] Bhat et al also showed improved plaque control and gingival index scores and reduction in S mutans counts with Mangifera indica mouth wash but less as compared to 0.2% Chlorehexidine mouth rinse[40].

Intra group comparison showed that there was statistically significant difference with the decrease in S mutans counts and improvement in plaque control and gingival scores in all the groups at different time intervals except for S mutans counts and plaque scores in saline group between day 15 and day 30 (Table 8 and 10). The results of the present study are in agreement with those of White and Armaleh, who showed that the efficiency of tongue scraping, saturated saline rinse and Listerine strips in reducing the salivary S mutans levels. They found that all the treatment groups showed a significant reduction in colony counts from baseline to one or more post treatment period.[41]

Acceptance of Nutmeg mouthwash in our study was excellent with 93.33% liking the taste and 80% of children willing to use in future as compared with 46.6% liking the taste and 33% willing to continue the use in saline group and 20% of children liking and willing to use 0.2% Chlorhexidine mouth wash.

Conclusion

Regular oral hygiene practices bring significant improvement in oral health of individuals.

Myristica fragrans mouth rinse was very well accepted by children and brought about comparable improvement in plaque control, gingival index scores and reduction in salivary S mutans counts as compared with 0.2% Chlorhexidine.

Hence Myristica fragrans mouth wash can be used as a suitable alternative to 0.2% Chlorhexidine in children.

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Tables and Figures

Table 1

Multiple comparison of mean difference in CFUs between study groups at different time intervals using Mann Whitney Post hoc Analysis

Time	(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
				Lower	Upper	
30 mins	CHX	Nutmeg	-53.27	-97.65	-8.88	0.02*
		Saline	-93.13	-137.52	-48.75	<0.001*
	Nutmeg	Saline	-39.87	-84.25	4.52	0.09
15 days	CHX	Nutmeg	-43.73	-78.18	-9.29	0.01*
		Saline	-105.07	-139.51	-70.62	<0.001*
	Nutmeg	Saline	-61.33	-95.78	-26.89	<0.001*
30 days	CHX	Nutmeg	-30.47	-66.19	5.26	0.11
		Saline	-112.47	-148.19	-76.74	<0.001*
	Nutmeg	Saline	-82.00	-117.72	-46.28	<0.001*

*- Statistically significant

Table 2

Comparison of mean CFUs between 3 groups at different time intervals using Kruskal Wallis Test

Time	Groups	N	Mean	SD	Min	Max	P-Value
Baseline	CHX	15	240.40	48.37	180	309	0.99
	Nutmeg	15	237.67	39.57	200	301	
	Saline	15	238.67	81.14	180	500	
30 mins	CHX	15	127.73	31.33	94	170	<0.001*
	Nutmeg	15	181.00	17.96	154	209	
	Saline	15	220.87	78.78	156	480	
15 days	CHX	15	100.87	20.19	78	140	<0.001*
	Nutmeg	15	144.60	22.38	100	180	
	Saline	15	205.93	60.12	143	400	
30 days	CHX	15	91.53	16.78	70	122	<0.001*
	Nutmeg	15	122.00	17.98	103	162	
	Saline	15	204.00	65.26	130	412	

*- Statistically significant

Table 3

Comparison of mean Plaque Index scores between 3 groups at different time intervals using Kruskal Wallis Test

Time	Groups	N	Mean	SD	Min	Max	P-Value
Day 1	CHX	15	1.30	0.36	0.8	1.8	0.60
	Nutmeg	15	1.40	0.29	1.0	2.0	
	Saline	15	1.27	0.40	0.6	2.2	
15 days	CHX	15	0.75	0.22	0.4	1.2	0.001*
	Nutmeg	15	0.89	0.15	0.6	1.2	
	Saline	15	1.14	0.37	0.5	2.0	
30 days	CHX	15	0.59	0.17	0.4	1.0	<0.001*
	Nutmeg	15	0.71	0.14	0.5	1.0	
	Saline	15	1.08	0.35	0.5	1.6	

*- Statistically significant

Table 4

Multiple comparison of mean difference in PI Scores between study groups at different time intervals using Mann Whitney Post hoc Analysis

Time	(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
				Lower	Upper	
15 days	CHX	Nutmeg	-0.14	-0.37	0.09	0.32
		Saline	-0.39	-0.63	-0.16	0.001*
	Nutmeg	Saline	-0.25	-0.49	-0.02	0.03*
30 days	CHX	Nutmeg	-0.11	-0.33	0.10	0.40
		Saline	-0.49	-0.70	-0.27	<0.001*
	Nutmeg	Saline	-0.37	-0.59	-0.16	<0.001*

*- Statistically significant

Table 5

Comparison of mean Gingival Index scores between 3 groups at different time intervals using Kruskal Wallis Test

Time	Groups	N	Mean	SD	Min	Max	P-Value
Day 1	CHX	15	1.89	0.21	1.6	2.3	0.53
	Nutmeg	15	2.01	0.18	1.8	2.4	
	Saline	15	1.93	0.43	1.6	3.0	
15 days	CHX	15	0.91	0.22	0.5	1.2	<0.001*
	Nutmeg	15	1.03	0.17	0.8	1.3	
	Saline	15	1.75	0.35	1.2	2.5	
30 days	CHX	15	0.38	0.21	0.0	0.6	0.001*
	Nutmeg	15	0.90	1.41	0.4	6.0	
	Saline	15	1.66	0.39	1.2	2.5	

*- Statistically significant

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Table 6

Multiple comparison of mean difference in GI Scores between study groups at different time intervals using Mann Whitney Post hoc Analysis						
Time	(I) Groups	(J) Groups	Mean Diff. (I-J)	95% CI for the Diff.		P-Value
				Lower	Upper	
15 days	CHX	Nutmeg	-0.11	-0.34	0.12	0.46
		Saline	-0.84	-1.07	-0.61	<0.001*
	Nutmeg	Saline	-0.73	-0.96	-0.50	<0.001*
30 days	CHX	Nutmeg	-0.52	-1.28	0.24	0.23
		Saline	-1.28	-2.04	-0.52	0.001*
	Nutmeg	Saline	-0.76	-1.52	0.00	0.04*

*- Statistically significant

Table 7

Comparison of mean CFUs between different time intervals in each study group using Friedman's Test							
Groups	Time	N	Mean	SD	Min	Max	P-Value
CHX	Baseline	15	240.40	48.37	180	309	<0.001*
	30 mins	15	127.73	31.33	94	170	
	15days	15	100.87	20.19	78	140	
	30days	15	91.53	16.78	70	122	
Nutmeg	Baseline	15	237.67	39.57	200	301	<0.001*
	30 mins	15	181.00	17.96	154	209	
	15days	15	144.60	22.38	100	180	
	30days	15	122.00	17.98	103	162	
Saline	Baseline	15	238.67	81.14	180	500	<0.001*
	30 mins	15	220.87	78.78	156	480	
	15days	15	205.93	60.12	143	400	
	30days	15	204.00	65.26	130	412	

*- Statistically significant

Table 8

Multiple comparison of mean difference in CFUs between different time intervals in each study group using Wilcoxon Signed Rank Post hoc Analysis						
Groups	BL vs 30 min	BL vs 15D	BL vs 30D	30min vs 15D	30min vs 30D	15D vs 30D
CHX	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Nutmeg	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Saline	0.001*	0.001*	0.001*	0.001*	0.001*	0.20

Table 9

Comparison of mean Plaque Index scores between different time intervals in each study group using Friedman's Test							
Groups	Time	N	Mean	SD	Min	Max	P-Value
CHX	Day 1	15	1.30	0.36	0.8	1.8	<0.001*
	15 Days	15	0.75	0.22	0.4	1.2	
	30 Days	15	0.59	0.17	0.4	1.0	
Nutmeg	Day 1	15	1.40	0.29	1.0	2.0	<0.001*
	15 Days	15	0.89	0.15	0.6	1.2	
	30 Days	15	0.71	0.14	0.5	1.0	
Saline	Day 1	15	1.27	0.40	0.6	2.2	<0.001*
	15 Days	15	1.14	0.37	0.5	2.0	
	30 Days	15	1.08	0.35	0.5	1.6	

*- Statistically significant

Table 10

Multiple comparison of mean difference in PI scores between different time intervals in each study group using Wilcoxon Signed Rank Post hoc Analysis			
Groups	D1 vs 15D	D1 vs 30D	15D vs 30D
CHX	0.001*	0.001*	0.004*
Nutmeg	0.001*	0.001*	0.001*
Saline	0.002*	0.003*	0.07

*- Statistically significant

Table 11

Comparison of mean Gingival Index scores between different time intervals in each study group using Friedman's Test							
Groups	Time	N	Mean	SD	Min	Max	P-Value
CHX	Day 1	15	1.89	0.21	1.6	2.3	<0.001*
	15 Days	15	0.91	0.22	0.5	1.2	
	30 Days	15	0.38	0.21	0	0.6	
Nutmeg	Day 1	15	2.01	0.18	1.8	2.4	<0.001*
	15 Days	15	1.03	0.17	0.8	1.3	
	30 Days	15	0.90	1.41	0.4	6.0	
Saline	Day 1	15	1.93	0.43	1.6	3	0.001*
	15 Days	15	1.75	0.35	1.2	2.5	
	30 Days	15	1.66	0.39	1.2	2.5	

*- Statistically significant

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Table 12

Multiple comparison of mean difference in GI scores between different time intervals in each study group using Wilcoxon Signed Rank Post hoc Analysis			
Groups	D1 vs 15D	D1 vs 30D	15D vs 30D
CHX	0.001*	0.001*	0.001*
Nutmeg	0.001*	0.01*	0.01*
Saline	0.008*	0.004*	0.04*

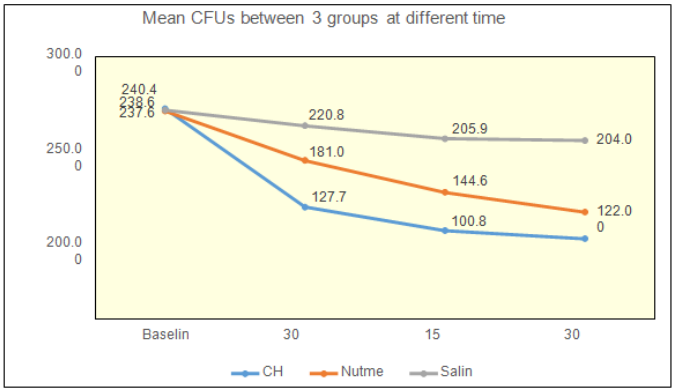
*- Statistically significant

Table 13: Patient compliance for different Mouth rinse

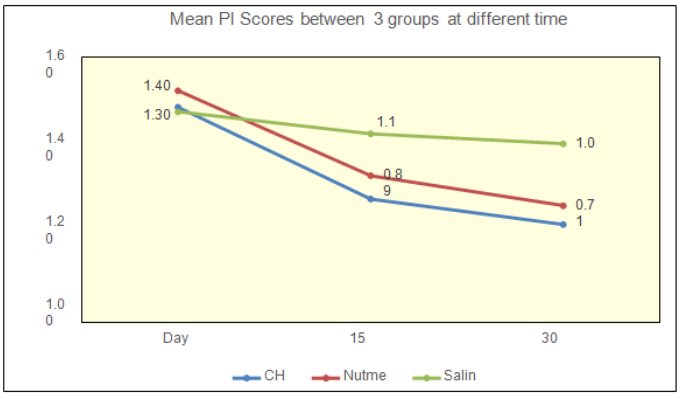
Total No. of patients	Group 1 (Chlorhexidine group) (n=15)	Group 2 (Nutmeg group) (n=15)	Group 3 (Saline group) (n=15)
Like the taste	n=03 (20%)	n=14 (93.33%)	n=12 (80%)
Like to Continue the use	n=03 (20%)	n=12(80%) n=2 (13.33%) Not willing n=1 (6.66%)	n=7(46.66%) n=5(33.33%) Not willing n=3(20%)

*- Statistically significant

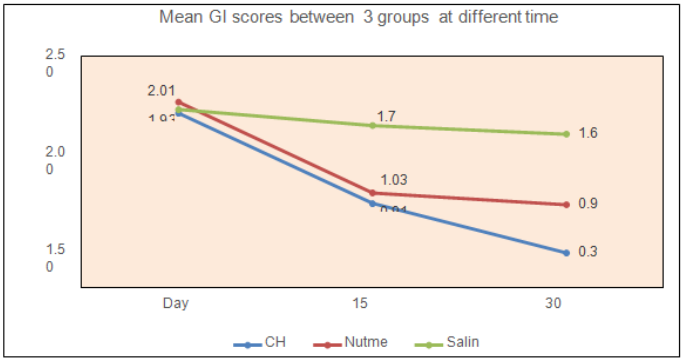
Graph 1



Graph 2



Graph 3



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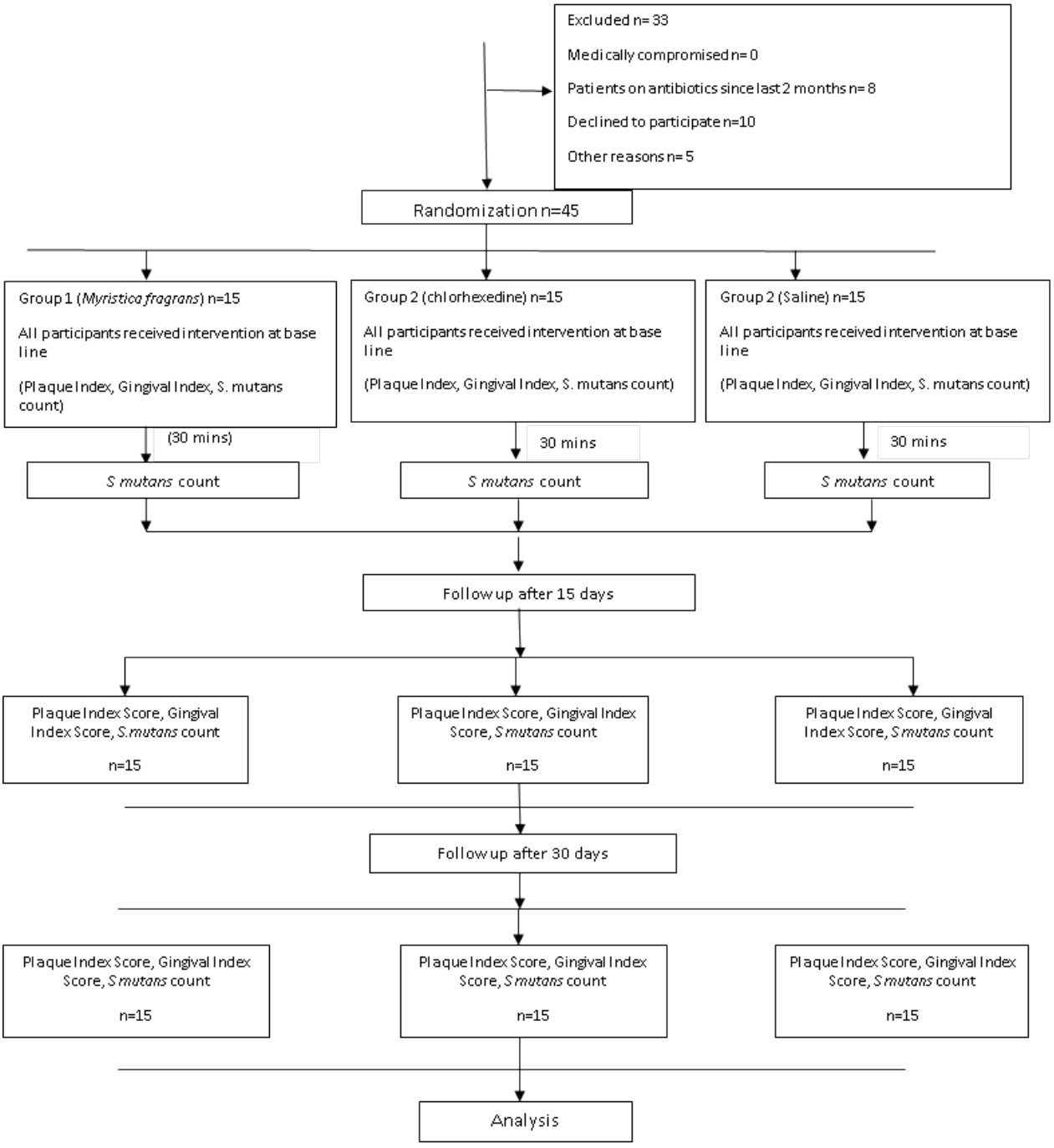
Flow Chart



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