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Research Article

COMPARATIVE EVALUATION OF ANTIBACTERIAL EFFICACY OF MYRISTICA FRAGRANS (1%), A NOVEL MOUTH WASH WITH CHLORHEXIDINE (0.2%) MOUTH WASH ON SALIVARY STREPTOCOCCUS MUTANS COUNTS: AN IN VIVO, PARALLEL GROUP PILOT STUDY

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A B S T R A C T

Aim: To do comparative evaluation of antibacterial efficacy of Myristica Fragrans (1%) a novel mouth wash with chlorhexidine (0.2%) mouth wash against salivary Streptococcus Mutans count.**Methods:** An experimental, in-vivo, parallel group randomized control trial was planned. School going children in the age group of 14-16 years was considered for the study. Twenty four students were selected to take part in the study. Twelve students were randomly allocated to each of the groups. Freshly prepared 1% nutmeg mouthwash and commercially available 0.2%chlorhexidine was used in the study. **Results:** The streptococcus mutans levels was significantly reduced in both 1% nutmeg mouthwash and in 0.2% chlorhexidine groups (p=0.003). The results showed that the two groups were comparable antibacterial properties against streptococcus mutans (p=0.92). **Conclusion:** The results showed that the 1% nutmeg herbal mouthwash is an effective agent against streptococcus mutans as chlorhexidine mouth wash after eight days.

INTRODUCTION

Good oral health is an integral component of good general health. Despite great improvements in the global oral health status. Dental caries still remains one of the most prevalent diseases. Oral micro-organisms are considered crucial for the initiation and progression of dental caries.[10]The early stage of dental caries is characterized by destruction of superficial dental structures caused by acids which are by-products of carbohydrate metabolism by Streptococcus mutans, a cariogenic bacterium. Colonization of teeth by cariogenic bacteria is one of the most important risk factors in the development of dental diseases. So plaque control represents the cornerstone of good oral hygiene practice.[17]

The tools most commonly used in mechanical supragingival plaque control are the toothbrush (manual or electric), floss, wood sticks, and interdental brushes. Despite the availability of various oral hygiene devices, even the most meticulous patient will not always completely remove all plaque. Evidence indicates that the degree of motivation and skill required for the effective use of these oral hygiene products may be beyond the ability of the majority of patients.[7]For these patients, a chemical plaque control approach is desirable as an adjunctive and can deal with the potential deficiencies of daily self-performed oral hygiene. This is where mouthwash comes to the picture.

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A mouthwash is a medicated liquid which is held in the mouth and swished by the the action of perioral musculature for a prescribed time. chlorhexidine gluconate, a bisbiguanide is considered to date the most effective anti-plaque agent.[14] But, it is not a 'Magic Bullet' and it also comes with certain side-effects, notably, tooth staining, taste disturbance, enhanced supragingival calculus formation and less commonly, desquamation of the oral mucosa.[15,3] Today's dentists are practicing in an era where the patients are more concerned about both their oral health and their overall medical wellbeing. Thus, in the midst of growing evidence of the connection between oral health and whole body health. Herbal medicines with their 'naturally occurring' active ingredients offer a gentle and enduring way for the restoration of health by the most trustworthy and least harmful way. Herbal medicine is both promotive and preventive in its approach. It is a comprehensive system, which uses various remedies derived from plants and their extracts to treat disorders and to maintain good health.[8]

In past, a large number of chemical agents have been discovered or synthesized in order to treat and cure these infections. Widespread and indiscriminate use of these drugs has led to the development of many drug-resistant strains which constitutes a major problem worldwide. Consequently, there is a need to look for alternatives for these products. Myristica fragrans (Nutmeg) is one of the native Indonesian popular spices which is also cultivated in parts of southern India. Nutmeg flesh made into sweets and syrup and essential oils. Nutmeg is widely used as spices and in alternative

medicine.[16]It is reported to have antidiarrheal, anti-inflammatory, anti-microbial and anti-cancer properties. Despite its medicinal properties, it has not been comprehensively evaluated for its antimicrobial potential against oral pathogens. The antimicrobial property may be attributed to the active phytochemicals in the nutmeg. The main active ingredient in nutmeg is *Macelignan* and is found to be active against both gram positive and gram negative organisms.[2]

Among all extracts tested, ethanolic extract of flesh was found to have the highest significant inhibitory effect against Gram-positive and Gram-negative bacteria and also highest bactericidal effects.[16,18] Previous in-vitro studies reported that a 1% (10mg/ml) of ethanolic extract of the flesh of Myristica fragrans to be effective against oral pathogens.[18]

Exploration of the literature revealed no in-vivo studies to evaluate the effect of *Myristica Fragrans* flesh essential oil mouth wash on oral pathogens. Hence a study was planned with the aim to compare the antibacterial efficacy of 1% *Myristica fragrans* ethanolic extract as mouthwash and 0.2% Chlorhexidine mouth wash on salivary Streptococcus Mutans. The hypothesis tested was that there is a difference in antibacterial efficacy between the two types of mouthwash when tested against Streptococcus Mutans.

MATERIALS AND METHODS

An experimental, in-vivo, parallel group (between subjects) triple blind randomized control trial was planned with an allocation ratio of 1:1.School going children in the age group of 14-16 years were considered for the study. The ethical approval was obtained from the Institutional Review Board of Bapuji Dental College and Hospital, Davangere. The study was planned in accordance with the CONSORT guidelines. As per the exploration of the literature available, this is the first in-vivo study using *Myristica fragrans* as a mouth wash. So as imprecise variance cannot be taken from in-vitro studies for sample size estimation. Hence, a sample size of 12 subjects per group was be taken considering it as a pilot trial. So, a minimum sample size of 12 subjects per treatment arm was taken. The total sample size of 12 x 2 (groups) = 24 was taken according to thumb rule by Julious (2005).[4]The power of the study was set at 80% and the significance level was set at $p \leq 0.05$.

Method of Preparation of the Nutmeg (1%) Mouth Wash

Fresh fruits of Nutmeg was be procured from courtyards of Kerala state, Flesh of the nutmeg was be dried in sunlight till it becomes easy to make a powder (crispy). The dried fruit fleshes were then powdered finely. Finely powdered flesh was then macerated with 100% ethanol. It was then subjected to filtration with Whatman filter paper to obtain a clear filtrate. The filtrate so obtained was reduced at a low temperature of less than 60^0 C to obtain a solid residue of the fruit extract and is diluted with distilled water to get 1% solution. Coloring agent was be added to match with chlorhexidine mouthwash for the purpose of blinding.[1]

Inclusion Criteria

Participating school students were with good general health with 14-16 years of age. Who are willing to give assent and also parents' consented to participate in the study. Students ready to comply with the appointment schedule and with at least one cavitated active caries lesion. Having a salivary Streptococcus Mutans count equal to or more than 10^5 CFU/ ml of saliva

Exclusion Criteria

Students who were not willing to give consent. Students with history of any systemic diseases/conditions, fibrotic gingival enlargement, using removable prostheses or an orthodontic appliance of any kind or any other oral condition which is conducive for over accumulation of plaque than normal. Those volunteers who had used medication such as antibiotics or mouthwash for five consecutive days or corticosteroids in the past 30 days before the start of the study. Those subjects who had a history of sensitivity to any mouthwash. Those who have undergone professional measures to remove plaque and calculus 15 days prior to the study. Students who are already using a mouth wash

Method of Assessment of Carious Lesion

To assess for the caries lesion, the child were seated comfortably on a chair with back support. The tooth suspected to have a carious lesion was confirmed by removing the overlying debris using a chip blower followed by examination using mouth mirror and CPITN Probe using adequate natural light. Tooth is considered as carius if the lesion is in pit and fissure, or on smooth tooth surface has an unmistakable cavity, undermined enamel or a detectable softened floor or wall. (WHO, Basic Oral Health Survey 2013).[12] A single examiner was calibrated to check for a carious lesion in the clinical setting before patient assessment.

For baseline assessment of Streptococcus mutans, 1ml of unstimulated saliva was be collected in a sterile test tube and assessed for Streptococcus mutans count. Subjects are asked to refrain from eating one hour before the collection of saliva. Unstimulated whole saliva is collected during the school hours(between 9-10 am). The children are asked to bend down and let saliva collect in the floor of the mouth for one minute. Then they were asked to expectorate into a sterile test tube. This process is repeated until the 1ml of saliva is obtained. The samples were then sealed labeled, coded and transported to the laboratory (Oral Pathology Department of Bapuji Dental College and Hospital, Davangere) for microbial analysis and is processed immediately.

Participants selected using the inclusion and exclusion criteria were allotted into the two groups randomly. Random assignment of the participants to the two interventional groups was done by a person other than the chief investigator using a computerized randomization technique. Randomization sequence is preserved and concealed in opaque envelops. The investigator the participants and the stastaitcian were blinded about the intervention.

Saliva samples were homogenized manually by stirring. Hundred micro litres of saliva were diluted with 1 ml of saline (1:10 dilution). Using an inoculation loop (2mm in diameter) 5 micro litre of 1:10 dilution sample were streaked on Mitis Salivarius bacterium (SMB) agar, a selective medium for Streptococcus Mutans. This was be incubated for 48 hours at 37^0 C in an atmosphere of 95% nitrogen and 5% carbon dioxide. After 48 hours of the incubation period, Streptococcus mutans appeared on the culture plate as a small rough raised

and adherent colonies and was confirmed by mannitol and sorbitol test. These colonies were counted by using an electronic colony counter (Deep Vision Company, India). As 5 microlitres of saliva sample were taken for culturing the number of colonies from this culture was multiplied by **1/5 x 10^3**and corrected for dilution factor to calculate the number of colonies for 1 ml of saliva. Subjects with greater than or equal to 10^5 CFU/ml was taken for the present study.

The respective mouthwashes were to be distributed to all the selected high school children. Each student was provided with a measuring cup. Children were instructed to rinse the mouth for 7 days with 10 ml of respective twice daily once after breakfast and before going to bed. Rinsing was done for one minute and then asked to expectorate it and not to rinse the mouth with water again for one hour. During the study period, the students were not asked to discontinue routine oral hygiene practices. They were also be instructed not to eat or drink anything for a minimum of half an hour after rinsing. A checklist was be given for assessing compliance for each subject (Annexure 1).

On the eighth-day subjects were instructed to rinse once after breakfast. One ml of unstimulated saliva samples are collected one hour after the subjects rinsed with the assigned mouth rinse and microbial analysis was done similar to the baseline assessment.

Statistical Analysis

The data obtained was compiled systematically in Microsoft Excel sheet and subjected to statistical analysis using SPSS software (Statistical Package for Social Sciences Software 20). The results were checked for normality of the data and the appropriate test was used for statistical analysis.

RESULTS

The data for the present experimental study was collected in the school setting. A total of 153 students were screened. 54 students were having the required inclusion criteria. From all the students who fitted the clinical inclusion criteria, an assent and an informed consent duly signed by their parents or guardians was obtained. 33 students gave consent for participation and baseline salivary samples were collected from them (Figure: 1). The samples were checked for the laboratory inclusion criteria related to S. Mutans count ($>10^5$ CFU).

In the age wise distribution of the participants out of 24 participants (Table:1). Eight (33%) subjects belonged to the age group of 14 years was in the nutmeg group and the rest were in the chlorhexidine group (21%). For the age group of 15 years, three (13%) were in the nutmeg group. Eight (33%) were in the chlorhexidine group. There were no participants in the age group of 16 years. In the gender wise distribution tables. There were five males in the nutmeg group and seven females. In the chlorhexidine group, there were nine males and three females. The mean score of the nutmeg group and chlorhexidine group at baseline was 364.08 ± 164.45 and 337.83 ± 124.24 respectively (Table 2). The maximum score was 650 for the nutmeg group and 579 for chlorhexidine. The minimum score was 158 for nutmeg and 126 for chlorhexidine at baseline. The mean score of the nutmeg group and chlorhexidine group at post-test were 77.27 ± 79.18 and 84.5 ± 101.54 respectively. The maximum score was 222 for the nutmeg group and 333 for chlorhexidine. The minimum score was zero for both. The results of the Kolmogorov-Smirnov test showed the data to be of non-normal distribution. So, the non-parametric test of significance was used in the present study. The within-group comparison was done using the Mann-Whitney U test. The between-group comparison was done using Wilcoxon signed rank test.The study results showed that there was no difference in the baseline values of the two groups after random allocation (Table: 3). Mean rank comparison between the two groups showed that the two groups were comparable with a p- value of 0.926 (Table: 4). Which shows that 1% nutmeg mouthwash is as effective as 0.2% CHX mouth wash. Wilcoxon signed rank test for Nutmeg group showed that there was a statistically significant difference between the baseline and post-test results within the group with a p-value of 0.003 for mean reduction of the salivary S.mutans count on the eight day(Table: 5)**.** Results obtained for the chlorhexidine group showed that there was statistically significant difference between the baseline and post test results within the groups with a p-value of 0.003 for mean reduction of the salivary S.mutans count on the eight day(Table: 6).

Twenty four students who met both the inclusion criteria participated in the study. One student in the chlorhexidine group discontinued due to nausea on using the mouth wash. Few of the subjects had alteration in taste after the use of the mouth wash which was present in both the groups.

Table 1 Frequency distribution age of the participants according to age and gender

GROUP	14 years	15 years	Males	Females
Nutmeg	8(33%)	3(13%)	5(21%)	7(29%)
Chlorhexidine	5(21%)	8(33%)	9(37%)	3(13%)

Table 2 Descriptive table for the baseline and post-test values of the two groups

	Mean	Median	Std. Deviation	Skewness	Std. Error of Skewness	Minimum	Maximum
Baseline nutmeg	364.0833	315.5	164.4514	0.52	0.637	158	650
Baseline CHX	337.8333	313	124.2401	0.218	0.637	126	579
Postnutmeg	77.2727	41	79.18976	1.001	0.661	0	222
PostCHX	84.5	39.5	101.5879	1.538	0.637	0	333

Table 3 Mean rank and Comparison of pretest baseline values between the two groups

	Groups	N	Mean rank	Sum of ranks	Mann-Whitney U	Z	Asymp. Sig. (2-tailed)
Base line	**Nutmeg**	12	13.00	156.00			
	CHX	12	12.00	144.00			0.729
	Total	24			66.00	-0.346	

Table 4 Mean rank and Comparison of post-test values between the two groups

	Groups	N	Mean Rank	Sum of Ranks	Mann-Whitney U	Z	Asymp. Sig. (2-tailed)
Post test	**CHX**	11	12.14	133.50			
	Nutmeg	12	11.88	142.50	64.500	-.093	0.926
	Total	23					

Table 5 Mean rank and Comparison of baseline and test values within the nutmeg group

Wilcoxon signed rank test for Nutmeg group					
	N	**Mean Rank**	**Sum of Ranks**	**Z**	**Asymp. Sig. (2-tailed)**
Negative Ranks	11[a]	6.00	66.00		
Positive Ranks	0[b]	.00	.00	-2.934[b]	0.003*
Ties	0[c]				
Total	11				

a. postnut<prenut b. postnut>prenut c. postnut = prenut *p≤ 0.05 is significant

Table 6 Mean rank and Comparison of baseline and test values within the chlorhexidine group

Wilcoxon signed rank test for Chlorhexidine Group					
	N	Mean Rank	Sum of Ranks	Z	Asymp. Sig. (2-tailed)
Negative Ranks	11[d]	7.00	77.00		
Positive Ranks	1[e]	1.00	1.00	-2.981[b]	0.003*
Ties	0[f]				
Total	12				

d. postchx<prechx e. postchx>prechx f. postchx = prechx *p≤ 0.05 is significant

*The Wilcoxon signed rank test results showed that there was significant difference between the baseline and the post-test rank values within the groups.

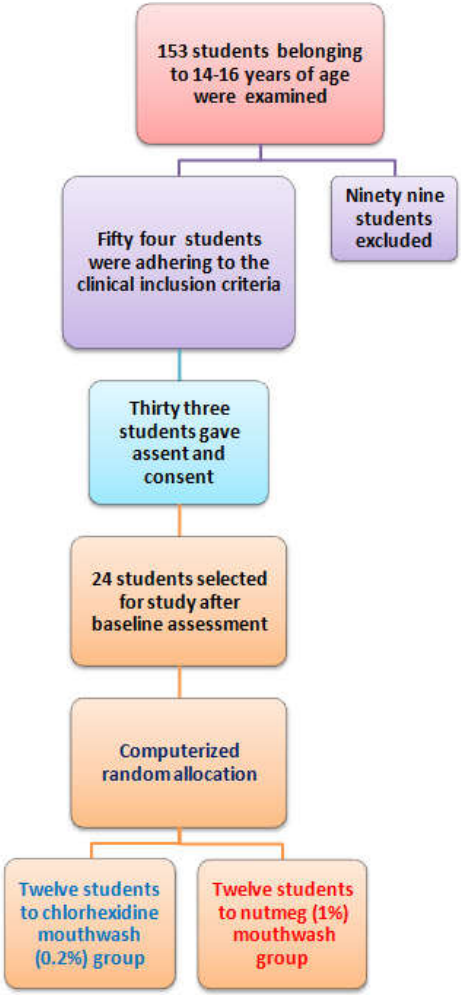

Figure 1 Showing the method of recruitment of participants.

DISCUSSION

The present study compared the antibacterial efficacy of two different mouth washes. The study results showed that there is significant reduction in streptococcus mutans count in the 1% nutmeg, novel mouth wash group which was comparable to that of 0.2% chlorhexidine mouthwash. This was similar to the previous in vitro studies conducted to assess the effectiveness of the essential oils against s. mutans. 2% chlorhexidine mouthwash when used as a mouth wash was found to be effective in reducing the salivary streptococcus mutans count to a statically significant level. Similar results were found in previous studies conducted by Agarwal P *et al*(2010)[1], Menendez *et al*.[14] and Kulkarnri *et al*.[6]There are two formulations of chlorhexidine available 0.12% and 0.2% both formulations are found to be equally effective. But as per literature, greater volume has to be used (15ml) for the concentration of 0.12% and for 0.2%, 10ml of mouth wash is recommended.[16]The participants were asked to use 10 ml mouth wash for eight days for one minute in order to standardize the use of mouth wash for both the groups. So in the present study we have instructed the subject to use 10ml of mouth wash each time. Chlorhexidine was found to be effective in reducing the s. mutants count in similar age groups also in studies by Agarwal P *et al* (2010)[1] (14-15 years) and Kulkarni *et al* (2003)(12-14 years).

In the present study it was found that 1 % nutmeg mouthwash was effective in significantly reducing the salivary streptococcus mutants count (p=0.003).This results could not be compared with other studies as there were no previous studies which have reported the use of nutmeg oil extract in the form of a mouthwash. The previous studies accessible are in the in vitro design against Streptococcus mutans and various other oral bacteria and was found to be effective. There are many antimicrobial agents found in nutmeg oil. The main antimicrobial properties have been linked to one particular ingredient in the oil by the name *Macelignan*.[5]Which is known as the main active ingredient in nutmeg. *Macelignan* is found to be active against both gram positive and gram negative organisms. The present study looks in the effectiveness of 1% nutmeg mouthwash which is a novel research area. No other studies has been reported in literature for the use of this herb as a mouthwash. This could be a novel area of interest especially when thinking of preventive measures in rural areas where the spice is easily available. But further research may be required to look into potential side effects and to understand the exact mechanism of action of nutmeg mouthwash on oral microbiota.

When the two types of mouthwash were compared for their ability for reducing the salivary S. mutants count although there was a numerical difference, there was no statistically significant result was found. The results of this study cannot be compared with other studies as no studies have been reported in the literature which has tried nutmeg mouth wash with that of chlorhexidine. The studies available are for chlorhexidine and other mouthwashes have given comparable results. All the subjects except one subject in the chlorhexidine group used the mouthwashes for all the assigned eight days. One of the subjects had a nauseating feeling after the use of the chlorhexidine mouthwash. This is a well-known side effect while using chlorhexidine mouthwash.[4]Few of the subjects had experienced an alteration in taste sensation after using the mouthwashes. Dental caries is the single most common chronic disease of childhood. There are various preventive methods to prevent tooth decay. The best known strategy is the plaque control and the reduction in the number of the principal pathogen which is Streptococcus mutans.[3]The use of mouthwash to control plaque bacteria dates back around 5000 years when the Chinese recommended the use of a child's urine for the control of gingivitis.[5]

Mouthwashes can be used for various preventative and therapeutic purposes like to treat oral infections, to reduce

inflammation, decrease halitosis and to deliver fluoride locally for preventing caries. The use of mouthwash is usually based on anecdotal evidence rather than scientific evidence especially for over-the counter (OTC) products.[9]This may often lead to the use of an inappropriate product and incorrect mode of application, leading to a failed treatment outcome. The patient's ability to perform good mechanical oral hygiene practices, dental status, gingiva, and oral mucosa, other oral diseases (xerostomia), and the efficacy of mouthwash and its potential adverse effects should be taken into consideration before recommending a particular mouthwash.[16]

Several plants were reported for their many therapeutic and pharmaceutical virtues, especially antioxidant, anti-tumor, and anti-infectious activities. A big part of the world's population still relies on the benefits of food for the treatment of common illnesses.[17]These have been found to be more culturally acceptable also. These benefits are due to their big content of bioactive compounds. Since the introduction of antibiotics, there has been a tremendous increase in the resistance of diverse bacterial pathogens. This shift in susceptibility greatly affects the ability to successfully treat patients. Many spices have been tested for their antimicrobial properties. The Gram-positive bacteria are known to be more sensitive to the antimicrobial compounds in *spices* than Gram-negative bacteria. The extent of sensitivity varied with the isolates and environmental conditions imposed. Certain spices have a direct effect on the rate of fermentation by stimulating acid production in starter cultures. Phenols, alcohols, aldehydes, ketones, ethers, and hydrocarbons have been recognized as major antimicrobial components in spices.[16]

Nutmeg is one such medicinal plant which is also used as a spice in daily life. Nutmeg oil contains monoterpenes such as -pinene, camphene, -pinene, sabinene, myrcene, a-phellandrene, a-terpinene, Limonene, 1, 8-cineole, g-terpinene, Linalool, terpinen-4-ol, safrole, methyl eugenol and myristicin, as their active principles. Their mode of antimicrobial action is related to their ability to inactivate microbial adhesion, enzymes, and cell envelope proteins.[4]
The anti-microbial properties of nutmeg oil have been discovered in previous in vitro studies and it was found to be effective against S. mutans. It was proved in previous studies that ethanol crude extracts from flesh of *Myristica fragrans* exhibited good potential against oral pathogens. The antibacterial activities of the extracts against both Gram-positive cariogenic bacteria. Thus, *Myristicafragrans* should be considered having beneficial potential in dentistry as oral care products such as toothpaste and mouthwash. Of the extracts tested the ethanolic extract of nutmeg has been found to be most effective against oral pathogens.

The present study is having active control in the form of 2% chlorhexidine mouthwash. As all the participants in the study are having active caries lesions active control is more ethically sound. Chlorhexidine is the gold standard among the available mouthwashes and the most popular. Chlorhexidine has broad-spectrum antimicrobial activity. It is effective against both Gram-positive and Gram-negative bacteria including aerobes and anaerobes, yeasts, fungi, and lipid-enveloped viruses. It increases the permeability of cell membrane followed by coagulation of cellular macromolecules. It does not interact with any microbial enzymes or receptors and hence does not lead to resistance from organisms. It was also found to have good antibacterial properties against s mutans in previous studies.

The study was conducted in the field setting included a total of 24 students in two groups from a school in Davangere city. The selected participants were randomly assigned into two groups using computerized random allocation. To ensure control of confounders and to reduce type 1 error.

The study aimed to test the different mouthwash in real life situations so no change in the daily oral hygiene habits or the dietary patterns were advised.

In the present study, nutmeg extract was used in the form of mouthwash and investigated for efficacy on Streptococcus mutans. Exploration of the available literature showed that there was no in vivo study which explored the effect of nutmeg mouthwash on streptococcus mutants. The nutmeg extract was prepared by the investigator at 1% concentration with the reference from previous studies which showed the maximum zone of inhibition at 3.9ug/ml against Streptococcus mutants.
The study was on the pilot phase of the trial as it was the first of its type with nutmeg mouthwash. As the imprecise variance cannot be taken from in-vitro studies for sample size estimation. A sample size of 12subjects per group was taken considering the research project as a pilot study according to thumb rule by Julious AS (2005).[4] So, the total sample size was 24 (12x2 groups). There is a theoretical risk that the beneficial effects of the first treatment might carry over into the second treatment period and thereby confound the detection of treatment effects. The parallel-group design is more versatile in that a stable disease state is not a pre-requisite, and therefore, trials in newly diagnosed patients are possible. Multiple treatment limbs are also more practical. The duration of a parallel-group trial may be shorter because only one treatment period is involved.

The reason for choosing the age group between 14- 16 years was that the age group was easily accessible, usually gives better compliance. They are also the representative age group of 15 years recommended by WHO for the basic oral health survey.
The present study was a pilot study which used the 1% nutmeg mouthwash for the first time. So the sample size was not calculated scientifically and the Julius thumb rule for the sample size for the pilot study was used. This met that the study results cannot be generalized. The present study could not be compared with the previous study results as the present study was the first of its kind.

CONCLUSION

Considering the present study which has limited applicability due to the fact that it is a pilot study. There is a need for further long term studies to consider the use and manufacture of 1% nutmeg mouth wash. It is also known that nutmeg oil has a variety of anti-oxidant effect which is also an area of insert for future studies Furthermore the use of herbal or natural medicine can be an alternative especially in the Indian scenario where people are increasingly using them in their daily routine.

References

1. Moye ZD, Zeng L, Burne RA. Fueling the caries process: carbohydrate metabolism and gene regulation

by Streptococcus mutans. J Oral Microbiol. 2014 Sep 5; 6-8.
2. Shukla N, Saha S, Singh S. Effect of Chlorhexidine with Fluoride Mouthrinse on Plaque Accumulation, Plaque pH-A Double Blind Parallel Randomized Clinical Trial. J ClinDiagn Res. 2016 Jul;10(7):62-65
3. Malhotra R, Grover V, Kapoor A, Saxena D. Comparison of the effectiveness of a commercially available herbal mouthrinse with chlorhexidinegluconate at the clinical and patient level. J Indian Soc Periodontol. 2011 Oct; 15(4):349-52
4. Santos A. Evidence based control of plaque and gingivitis. J Clin Periodontol 2003; 30(Suppl.5):13-6.
5. Pizzo G, Cara ML, Licata ME, Pizzo I, Angelo MD. The effects of an essential oil and an amine fluoride/stannous fluoride mouthrinse on supragingival plaque regrowth. J Periodontol 2008; 79(11):77-83.
6. Jones CG. Chlorhexidine: is it still the gold standard?. Periodontology 2000. 1997 Oct 1;15(1):55-62.
7. Manipal S, Hussain S, Wadgave U, Duraiswamy P, Ravi K. The Mouthwash War-Chlorhexidine vs. Herbal Mouth Rinses: A Meta-Analysis. Journal of Clinical and Diagnostic Research: JCDR. 2016 May;10(5):81-87.
8. Gupta AD, Bansal VK, Babu V, Maithil N. Chemistry, antioxidant and antimicrobial potential of nutmeg (Myristicafragrans Houtt). *Journal of Genetic engineering and Biotechnology*. 2013 Jun 30;11(1):25-31.
9. Shafiei Z, Shuhairi NN, MdFazly Shah Yap N, Harry Sibungkil CA, Latip J. Antibacterial activity of Myristica fragrans against oral pathogens. J Evid Based *Complementary* Altern *Med*. 2012 Aug 28;2012:1-7
10. Singh A, Thakur R B, Singh V. Antimicrobial Susceptibility of Myristica fragrans Extract against Oral Pathogens. Int J Curr Microbiol Ap Sci. 2017 6(1): 339-343.
11. Agarwal P, Nagesh L, Murlikrishnan. Evaluation of the antimicrobial activity of various concentrations of Tulsi (Ocimum sanctum) extract against Streptococcus mutans: An in vitro study. *Indian Journal of Dental Research*. 2010; 21(3):357-59.
12. Julious AS. Sample size of 12 per group rule of thumb for a pilot study. Pharmaceutical Statistics. 2005; 4(4):287-91.
13. Oral health surveys – basic methods. 4th edition. Geneva: World Health Organization; 1997.
14. Nurjanah S, Putri IL, Sugiarti DP. Antibacterial Activity of Nutmeg Oil. KnE Life Sciences. 2017 Nov 26;2(6):563-9.
15. Keukenmeester RS, Slot DE, Rosema NA, Weijden GA. Determination of a comfortable volume of mouthwash for rinsing. *International journal of dental hygiene*. 2012 Aug 1;10(3):169-74.
16. Parashar A. Mouthwashes and their use in different oral conditions. Sch J Dent Sci. 2015;2(2):186-91.
17. Richens A. Proof of efficacy trials: cross-over versus parallel-group. Epilepsy research. 2001;45(1):43-7.
18. Kulkarni VV, Damle SG. Comparative evaluation of efficacy of sodium fluoride, Chlorhexidine and Triclosanmouthrinses in reducing mutans streptococci count in saliva: an in vivo study. J Indian SocPedodPrev Dent 2003;21(3):98–104.
19. Menendez A, Li F, Michalek SM, Kirk K, Makhija SK, Childers NK. Comparative analysis of the antibacterial effects of combined mouthrinses on Streptococcus mutans. Oral Microbiol Immunol 2005;20:31–4.

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