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Research Article

EVALUATION OF ANTIMICROBIAL EFFICACY OF 5% *MYRISTICA FRAGRANS* MOUTHWASH IN GINGIVITIS SUBJECTS - A CLINICO MICROBIOLOGICAL STUDY

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ABSTRACT

Gingivitis is the inflammation or infection of gingiva that support the teeth. The prevailing belief regarding the cause for gingivitis is the toxins produced by the bacteria in the mouth, particularly the bacteria that causes the formation of plaque. *Myristica fragrans* commonly known as nutmeg possesses anti-carcinogenic, anti-inflammatory activities, antioxidant effect, anti-diabetic, and antibacterial properties. The seeds of nutmeg have the ability to inhibit the growth of Gram Negative and Positive bacteria. **Objective:** 1. To evaluate clinical parameters at baseline and 21 days post scaling after the use of *Myristica fragrans* mouthwash. 2. To evaluate the colony count of *Porphyromonas gingivalis* in plaque samples at baseline and 21 days after the use of *Myristica fragrans* mouthwash. **Methodology:** A total of 40 gingivitis subjects were selected and divided randomly into Group A and B. Group A, non-surgical periodontal therapy followed by *Myristica fragrans* mouthwash (20) and Group B, non-surgical periodontal therapy alone (20). The supragingival plaque samples were collected at baseline and 21 days after non surgical periodontal therapy. **Results:** Significant reduction in clinical parameters and colony count of *Porphyromonas gingivalis* after the use of 5% *Myristica fragrans* mouthwash.

INTRODUCTION

The inflammatory clinical condition of the gingival tissue which commonly termed as gingivitis is now one of the most common diseases in the oral cavity. The condition is restricted to the soft-tissue area of the gingival epithelium and connective tissue. There are various forms of gingivitis based on clinical appearance, duration of infection, severity, and etiology. The prevailing belief regarding the cause for gingivitis is the bacteria that cause the formation of dental plaque. Plaque is a thin film that forms on the tooth surface due to poor oral hygiene. As plaque harbors a large number of bacteria, inflammation can occur in the gingival tissue.[1,2]

The initial symptoms of gingivitis are not obvious, and bleeding on probing and redness of the gums do not occur until early lesions of gingivitis are present.

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It is usually painless, rarely causes spontaneous bleeding, and its clinical symptoms are not obvious enough for most patients to recognize the disease.[3]

Some of the bacteria that cause the oral infections includes Streptococci spp. (including S. mutans), Porphyromonas spp., Actinobacillus spp., Bacteroides spp., Staphylococci spp., Fusobacterium nucleatum, Veillonella parvula, Actinomyces naeslundii, and Porphyromonas gingivalis. Formation of dental plaque and lack of maintenance of oral hygiene is the main reasons for the progression of oral disease.[4]

Myristica fragrans commonly known as nutmeg has four parts - The skin, the fruit, the seed and the mace, where all the parts have different properties. The mace and seed are used are used as spices, while the nutmeg oil is used in some medicinal purposes. The main constituents of *Myristica fragrans* have been found to be alkyl benzene derivatives, terpenes, alpha-pinene, beta-pinene, myristic acid, neolignan and macelignan. Nutmeg mainly possess anti inflammatory, anti bacterial and anti oxidant properties, hence it will act against some harmful bacteria. According to some

studies the nutmeg seed had strong anti-cariogenic and antibacterial effect against oral microorganisms such as streptococcus species, and lactobacillus species, actinomyces viscosus, porphyromonas gingivalis and staphylococcus aureus.[5,6]

Some of the plaque control measures include the use of toothpastes, mouthwash, sprays and irrigants, chewing gums or varnishes. But the most effective method among these includes the use of mouthwash. Chlorhexidine is the most appropriate choice. But being the gold standard mouthwash, chlorhexidine has some disadvantages like staining of teeth, allergy and burning sensation. Hence the need for any herbal mouthwash is necessary which will be convenient for the patient.[7]

Several studies have highlighted the promising potential of *Myristica fragrans* against few Gram positive and Gram negative bacteria. Further clinical studies are needed to particularly show the efficacy of seed extract of *Myristica fragrans* as mouthwash. Therefore, we aimed to assess and compare the effect of *Myristica fragrans* seed extract mouthwash on various clinical parameters and also its antimicrobial effect on potent periodontal pathogen, *Porphyromonas gingivalis* in gingivitis subjects.

MATERIALS AND METHODS

A total of 40 subjects diagnosed with gingivitis aged 18-45 years, will be randomly selected from the Outpatient Department of Periodontics, P.M.N.M Dental College & Hospital, Bagalkot. Keeping alpha error at 5%, power of the study at 80%, the sample size estimated is approximately 16 in each group. For follow-up study, to avoid loss due to attrition we will be taking 20 subjects in each group. Ethical approval was taken from the institutional ethical committee for the study.

Subjects will be randomly divided into 2 groups

Group A: Non-surgical periodontal therapy followed by *Myristica fragrans* mouthwash.

Group B: Non-surgical periodontal therapy alone.

Preparation of the *Myristica fragrans* Mouth rinse [7,8]

Nutmeg seed was shade dried and made into powder. 50gm of powder was soaked in 2000ml of distilled water and heated at 1000°C for 30 minutes using a hot plate, filtered using Whatman No. 1 filter paper. The extract will then dried using hot air oven using 45°C. The dried extract is then triturated in a uniform direction using mortar and pestle to form a uniform mixture. Water was added to obtain the desired quantity of mouthwash. To this sodium saccharin as sweetening agent, sodium lauryl sulphate as surfactant, Sodium benzoate as preservative and menthol as coolant were added to prepare it to a final mouthwash. (figure no.1)

Powder **Heating** **Filtration** **Drying**

Figure 1: Showing the preparation of *myristica fragrans* mouthwash

Dried extract **Trituration** **Mouthwash**

The exclusion criteria includes patients with systemic diseases that could influence periodontal conditions, those who have undergone periodontal therapy in the past 6 months, patients on any medication, those who have any allergy to mouthwashes, subjects consuming tobacco in any form and pregnant or lactating females.

Clinical examination to assess the periodontal condition

Clinical examination will be performed on all the subjects using Simplified Oral Hygiene Index (John C. Greene and Jack R. Vermillion 1964), Gingival Index (Loe & Silness 1963), Plaque Index (Silness & Loe 1964) and Bleeding on probing (Muhlemann & Son, 1971).

All groups will follow same oral hygiene instructions except allocated mouthwashes. Clinical parameters will be recorded along with plaque samples collection on the day of examination and 21 days after *Myristica fragrans* mouthwash use. Oral prophylaxis will be carried out after baseline data collection and participants were instructed to use the given mouthwash and not to use any other oral hygiene aids except toothbrush and toothpaste. They will be instructed to rinse 10ml of assigned mouthwash twice a day. On day 0 and 21 supragingival plaque will be collected using sterile curette from the buccal surface of first molar in each quadrant and then it will be mixed in sterile eppendorf tube containing 500µl of reduced transport fluid (RTF).[9] The plaque samples will be subjected to bacterial culture to check the counts of P. gingivalis bacteria pre and post use of *Myristica fragrans* mouthwash.

Sample collection will be done by single investigator and analysis of plaque sample will be done by a microbiologist.

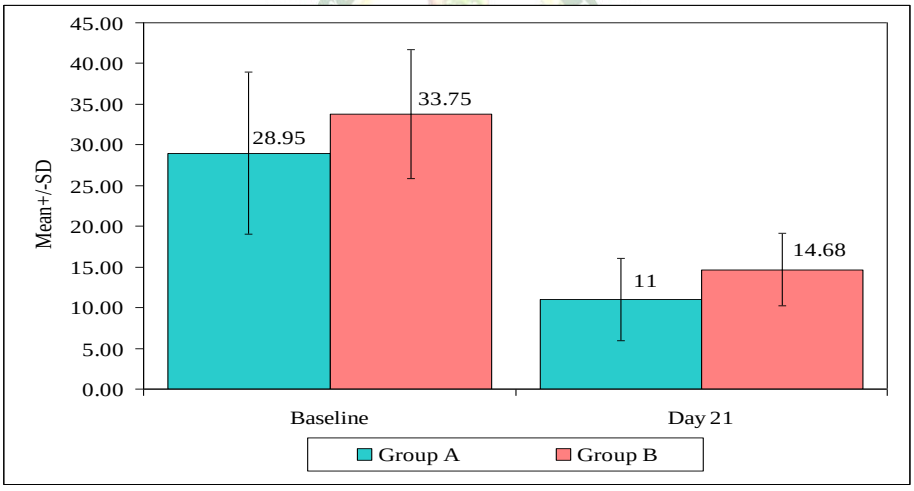
The data collected will be analysed using chi-square test, Kolmogorov Smirnov test, Independent t or Mann- Whitney U test, Dependent t or Wilcoxon matched pairs test. All the participants will be clearly explained regarding the need and design of the study. A duly signed written informed consent will be obtained from all the subjects willing to participate in the study.

RESULTS

The results of our study showed that there was a statistical difference in colony forming unit of P. gingivalis as well as the clinical parameters between both groups at day 21.

Table 1: Showing Comparison of Group A and Group B with CFU counts

Time points	Group A		Group B		t-value	p-value
	Mean	Std. Dev.	Mean	Std. Dev.		
Baseline	28.95	9.95	33.75	7.93	-1.6869	0.0998
Day 21	11.00	5.09	14.68	4.41	-2.3279	0.0260*
Difference	18.06	6.50	18.47	4.41	-0.2260	0.8225



Graph 1: Showing Comparison of Group A and Group B with CFU counts

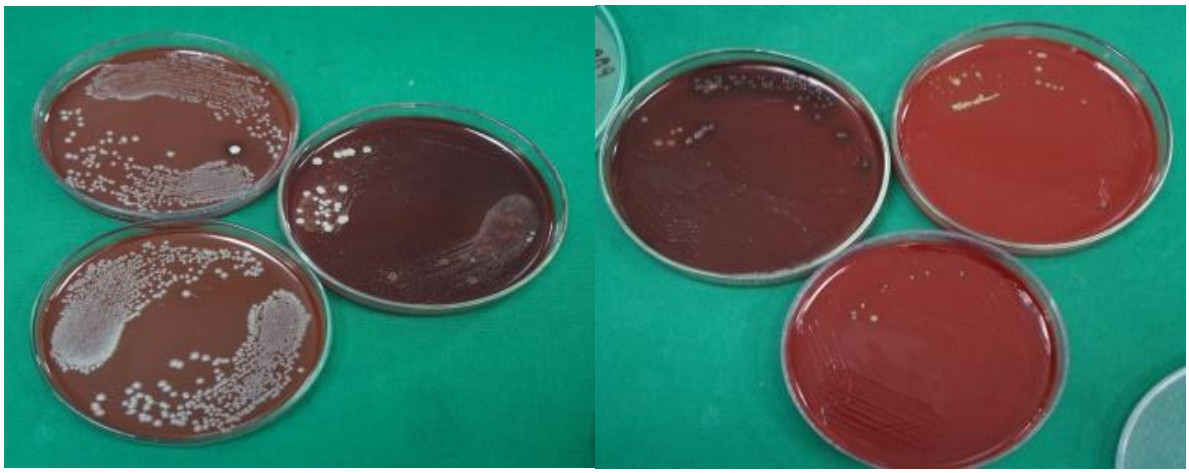


Figure 2: Showing bacterial culture of *P.gingivalis* at baseline and day 21 of group A

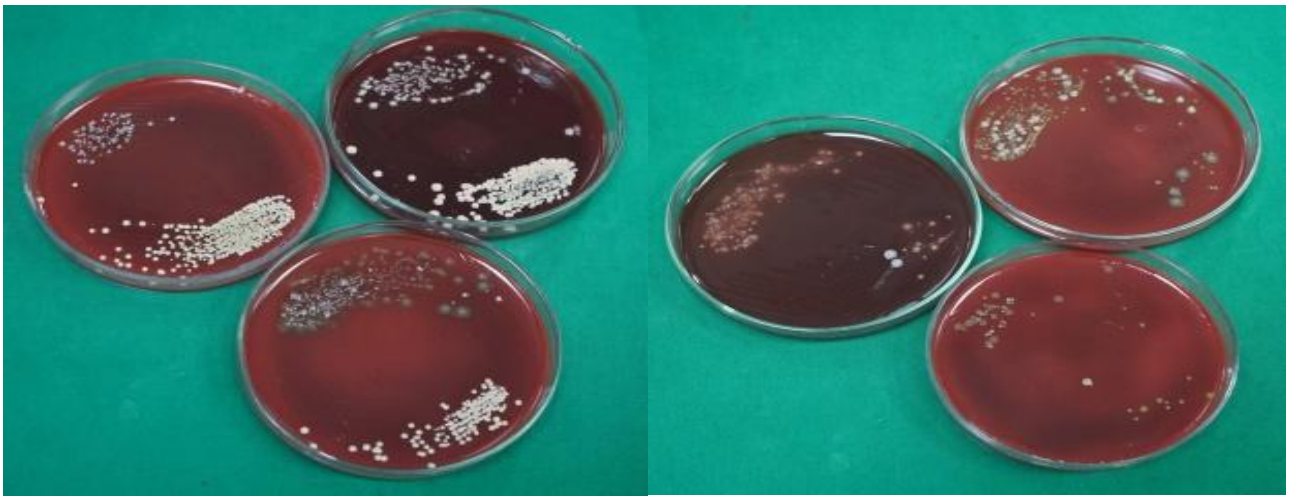
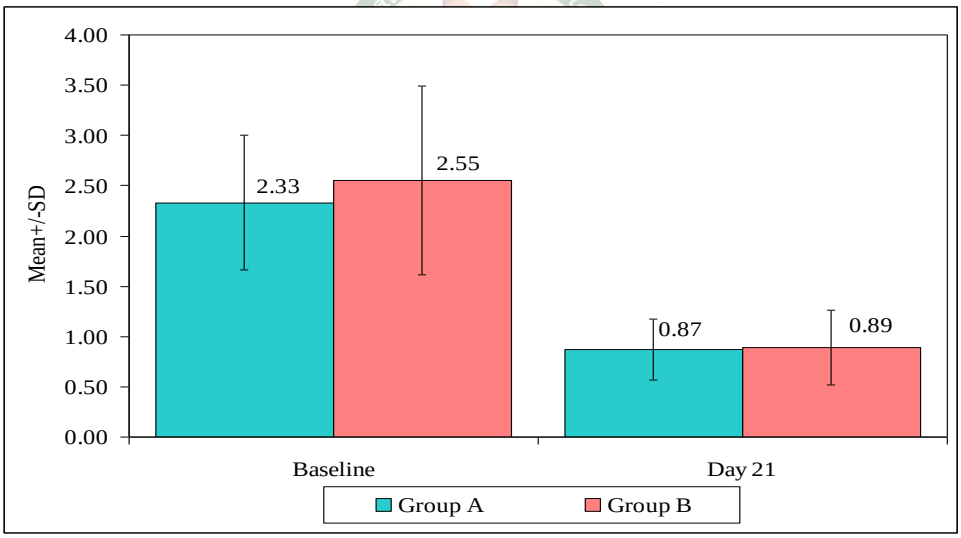


Figure 3: Showing bacterial culture of P. gingivalis at baseline and day 21 of group B

The group who received scaling along with *Myristica fragrans* mouthwash has got a significant reduction in P. gingivalis count compared to the other group who received on scaling. [Table 1. Graph 1]

Table 2: Showing Comparison of Group A and Group B with OHI-S scores at baseline and day 21 treatment

Time points	Group A		Group B		t-value	p-value
	Mean	Std. Dev.	Mean	Std. Dev.		
Baseline	2.33	0.67	2.55	0.94	-0.8712	0.3891
Day 21	0.87	0.30	0.89	0.37	-0.2124	0.8331
Difference	1.61	0.63	1.64	0.80	-0.1279	0.8990

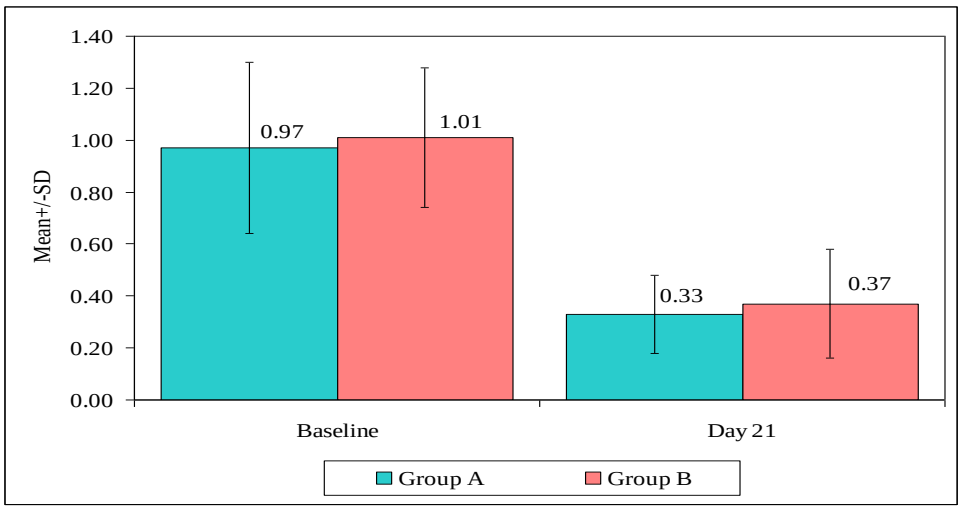


Graph 2: Showing comparison of Group A and Group B with OHI-S scores at baseline and day 21 treatment

The comparison of OHI-S score showed a mean difference of 1.61 in group A from base line to day 21 and group B showed a mean difference of 1.64 from base line to day 21. [Table 2, Graph 2]

Table 3: Showing Comparison of Group A and Group B with GI scores at baseline and day 21 treatment

Time points	Group A			Group B			U-value	Z-value	p-value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Baseline	0.97	0.33	20.18	1.01	0.27	20.83	193.5	-0.1623	0.8711
Day 21	0.33	0.15	17.56	0.37	0.21	19.34	145.5	-0.4912	0.6233
Difference	0.71	0.25	19.26	0.63	0.28	17.82	148.5	0.3961	0.6920

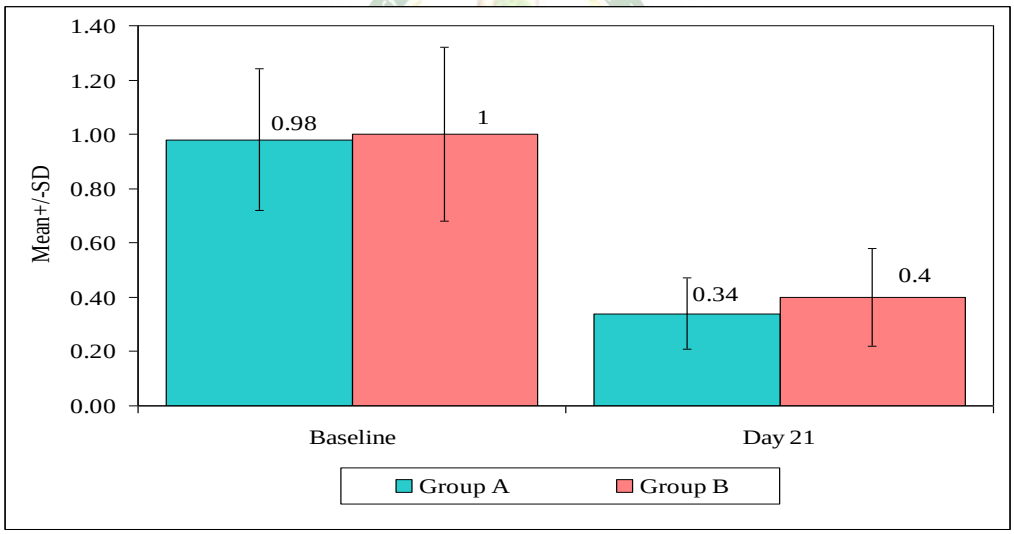


Graph 3: Showing Comparison of Group A and Group B with GI scores at baseline and day 21 treatment

Gingival index score showed a mean difference of 0.71 in group A from base line to day 21 and group B showed a mean difference of 0.63 from base line to day 21. [Table 3, Graph 3]

Table 4: Showing Comparison of Group A and Group B with PI scores at baseline and day 21 treatment

Time points	Group A			Group B			U-value	Z-value	p-value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Baseline	0.98	0.26	19.60	1.00	0.32	21.40	182.0	-0.4734	0.6359
Day 21	0.34	0.13	16.21	0.40	0.18	20.55	122.5	-1.2200	0.2225
Difference	0.72	0.20	21.06	0.59	0.26	16.21	118.0	1.3626	0.1730

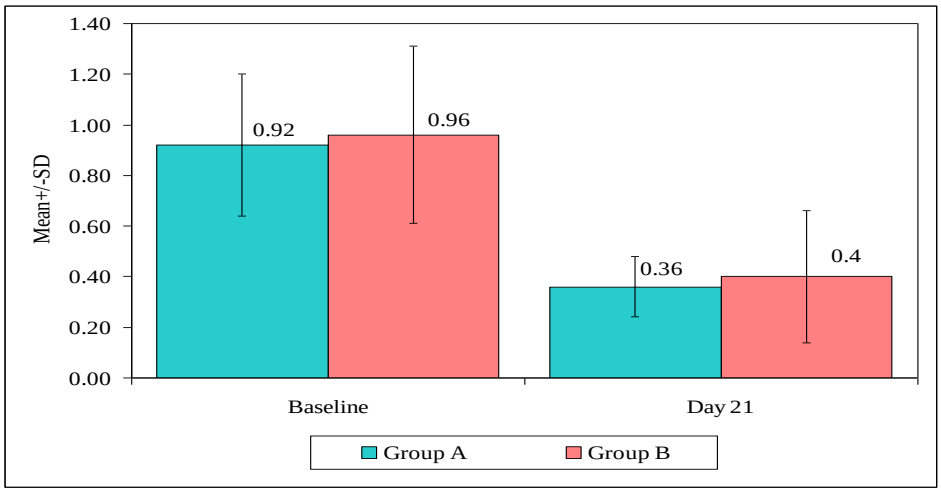


Graph 4: Showing Comparison of Group A and Group B with PI scores at baseline and day 21 treatment

Plaque index score showed a mean difference of 0.72 in group A from base line to day 21 and group B showed a mean difference of 0.59 from base line to day 21. [Table 4, Graph 4]

Table 5: Showing Comparison of Group A and Group B with BOP scores at baseline and day 21 treatment

Time points	Group A			Group B			U-value	Z-value	p-value
	Mean	SD	Mean rank	Mean	SD	Mean rank			
Baseline	0.92	0.28	19.18	0.96	0.35	21.83	173.5	-0.7033	0.4819
Day 21	0.36	0.12	18.24	0.40	0.26	18.74	157.0	-0.1268	0.8991
Difference	0.63	0.16	19.24	0.56	0.29	17.84	149.0	0.3803	0.7038



Graph 5: Showing Comparison of Group A and Group B with BOP scores at baseline and day 21 treatment

Bleeding on probing score showed a mean difference of 0.63 in group A from base line to day 21 and group B showed a mean difference of 0.56 from base line to day 21. [Table 5, Graph 5]

In the present study none of the participants complained of stained teeth or altered taste sensations throughout the study period.

DISCUSSION

The antibacterial activities of seed extract of *Myristica fragrans* mouthwash on gingivitis was investigated in this study.

Using a chemical plaque control agent (like a mouthwash) to supplement mechanical plaque removal can produce an antimicrobial effect throughout the oral cavity. CHX is one of the most commonly prescribed chemical plaque control agents in dentistry.[7] Ahmad et al., in their study, confirmed the effect of long-term use of CHX mouth rinse on increasing the dental stains, allergy, and burning mouth. In recent times, the use of herbal mouthwash is on the rise due to the spread in the awareness of the effect of complementary and alternative medicine.[10]

In another study Shafiei Z et al demonstrated the antibacterial activity of nutmeg against oral cavity pathogens. The results showed that the active compound trimyristin contained in nutmeg functions as an antibacterial against gram positive and negative bacteria.[11]

Twinkle Francis investigated the anti inflammatory and cytotoxicity of nutmeg based gel and the results showed that the nutmeg based gel has high potential in anti inflammatory activity and shows less cytotoxic activity.[12]

In our study all the plaque samples collected in RTF immediately brought to laboratory and centrifuged and diluted as 1:10. Culturing for *P. gingivalis* was done on brucella blood agar and incubated in CO_2 jar for 48-72 hrs. After the end of the incubation check for the growth and count the colonies to get the colony forming units (CFU/ml). In group A, the mean difference observed from baseline to day 21 is 18.06 with p-value of 0.0001 and in group B, the mean difference observed from baseline to day 21 is 18.47 with p-value of 0.0001. Similarly for OHI-S score, group A showed the mean difference 1.61 from baseline to day 21 with p-value of 0.0001 and group B showed the mean difference of 1.64 with p-value of 0.0001.

GI index score showed a mean difference of 0.71 in group A from base line to day 21 with p-value of 0.0003 and group B showed a mean difference of 0.63 from base line to day 21 with p-value of 0.0001. Similarly Plaque index score showed a mean difference of 0.72 in group A from base line to day 21 with p-value of 0.0003 and group B showed a mean difference of 0.59 from base line to day 21 with p-value of 0.0001. Bleeding on probing score showed a mean difference of 0.63 in group A from base line to day 21 with p-value of 0.0003 and group B showed a mean difference of 0.56 from base line to day 21 with p-value of 0.0003.

All the results indicate the statistical difference in the various clinical parameters among both the groups.

CONCLUSION

Herbal mouthwashes have a promising role in dentistry; having proper knowledge and their effects on teeth would prove them as a successful dental therapeutic agent. This study has explained the role of seed extract of nutmeg mouthwash in reducing various clinical parameters and the colony count of *Porphyromonas gingivalis*. Hence we can conclude that as *Myristica fragrans* mouthwash can be easily prepared and economical, it can be used as an adjunct to the mechanical periodontal therapy.

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