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Original Article

Dentistry Section

Comparative Evaluation of the Antimicrobial Efficacy and Abrasivity of a Herbal Dentifrice Formulated with *Myristica fragrans* and a Commercially Available Herbal Dentifrice: An In-vitro Study

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ABSTRACT

Introduction: Effective plaque control is important for proper oral health maintenance, and the toothbrush-dentifrice combination is essential for achieving this. Recently, herbal toothpastes have gained more popularity amongst people.

Aim: To formulate a novel herbal toothpaste containing *Myristica fragrans* (nutmeg) and compare its antimicrobial efficacy and abrasivity with a commercially available herbal toothpaste.

Materials and Methods: This in-vitro study was conducted on 24 non-carious permanent extracted teeth with intact coronal structure. These were equally divided into experimental and control groups (n=12). The nutmeg toothpaste was formulated using nutmeg powder, tulsi leaf powder, and clove powder as the main herbal ingredients. Dabur red was selected as the commercial herbal toothpaste. The zone of inhibition of both toothpastes was evaluated against *Streptococcus mutans* and *Lactobacillus* species using the agar well diffusion method. Enamel specimens measuring 5×5×5 mm were mounted on acrylic blocks and brushed for 28 days. Profilometric analysis was conducted on the 1st, 7th, and 28th day. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 23.0 software, and independent t-tests and Analysis of Variance (ANOVA) tests were used.

Results: 24 teeth were used. A total of 6 samples of toothpaste were considered for antimicrobial testing. The zone of inhibition for the nutmeg toothpaste and Dabur red was 28 mm and 32.17±0.42 mm, respectively, against both organisms. The increase in surface roughness of enamel was not statistically significant between the two groups (p>0.05).

Conclusion: Both toothpastes exhibited antimicrobial activity against the test microorganisms and significantly increased the surface roughness of enamel from baseline to the 28th day. The nutmeg toothpaste caused less enamel abrasion, but the difference was not significant.

Keywords: Dental plaque, Dentistry, Toothpastes

INTRODUCTION

Dental plaque is the primary aetiological factor of two of the most commonly encountered global oral health problems: dental caries and periodontal disease [1,2]. Thus, effective plaque control is an essential aid in the prevention of these diseases. The most proven technique for controlling plaque is meticulous mechanical plaque control combined with antiplaque and antibacterial agents [1,3]. This is accomplished by the toothbrush-dentifrice combination.

There are various commercial chemical toothpastes available in the market today. However, in recent years, the use of herbal products in toothpastes has gained more popularity among people. This can be attributed to the side effects, such as the risk of an allergic reaction from the chemical components, and the independent benefits offered by herbal alternatives [4,5]. Furthermore, this trend is supported by multiple studies that prove the equal effectiveness of incorporating these agents into oral health care products [5,6]. Additionally, the rise in disease incidence, pathogenic bacterial resistance to currently used antibiotics and chemotherapeutics, opportunistic infections in immunocompromised individuals, and financial concerns in developing countries have all contributed to the global need for alternative options for oral health care products that are safe, effective, and affordable [7].

Myristica fragrans, commonly known as nutmeg, has been used as a spice and flavouring agent in the food industry and domestically for years. Due to its intricate molecular structure, it has gained recognition for its many therapeutic benefits. As a result, it has the potential to be employed in dental care products as a natural antimicrobial agent [8]. Several herbal components have been studied in the scientific literature, except for nutmeg. Furthermore, there is a scarcity of research into modifying and improving the content of toothpastes for children.

Abrasives are an integral part of dentifrices. They remove food debris, stained pellicle, and plaque [1,9]. Therefore, to successfully implement mechanical plaque control, a certain level of abrasivity must be tolerated. However, abrasives can have adverse consequences on the dental hard tissues, enamel, and dentin if their use is not controlled. Highly abrasive toothpastes can dislodge occluded dentinal tubules and reduce enamel hardness [10]. This can cause hypersensitivity, recession, and discomfort for the patient. Moreover, this holds a special significance when it comes to deciduous teeth, as their enamel thickness is less than that of permanent dentition [11]. This may increase the risk of caries progression in children.

Nutmeg is known to be mildly abrasive on the skin, but studies have not been conducted to conclusively determine its efficacy on teeth. Thus, this study aims to formulate a herbal toothpaste containing *Myristica fragrans* (nutmeg) and compare its antimicrobial efficacy and abrasivity with a commercially available herbal toothpaste. The objectives are to formulate an herbal dentifrice using nutmeg powder, to compare and evaluate the antimicrobial efficacy of the dentifrice formulated with nutmeg and a commercially available

dentifrice against *Streptococcus mutans* and *Lactobacillus* spp., and to compare the abrasivity of the dentifrice formulated with nutmeg with a commercially available herbal dentifrice.

MATERIALS AND METHODS

This in-vitro study was conducted at the AISSMS College of Pharmacy, Pune, under the guidance of an expert in Pharmaceutics, and at Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Pune, Maharashtra, India, from June 2021 to September 2021, after obtaining ethical clearance from the Institutional Ethical Committee (EC/NEW/INST/2019/329).

Inclusion criteria: Non-carious permanent teeth with intact coronal structure were included for the profilometric analysis conducted in this study.

Exclusion criteria: Previously restored endodontically treated teeth and teeth with developmental anomalies were excluded for the profilometric analysis conducted in this study.

Sample size: The sample size for the antimicrobial testing was calculated to be six per group using the G Power software. The effect size was determined using the data obtained from a previous study conducted by Varghese S and Sapna B, [12].

- **Group-1** (n=6): Herbal dentifrice formulated with nutmeg (experimental group).
- **Group-2** (n=6): Commercially available herbal dentifrice (control group).

The sample size for the profilometric analysis was also calculated using the G Power software, with 24 participants per group. The effect size was determined using the data obtained from a previous study conducted by Kumar KK et al., [10].

- **Group-1** (n=24): Herbal dentifrice formulated with nutmeg (experimental group).
- **Group-2** (n=24): Commercially available herbal dentifrice (control group).

Procedure

A pilot study was conducted wherein three different formulations of the nutmeg toothpaste were developed based on a study by Gautam D et al., and modified based on the guidance provided by the expert in pharmaceutics [Table/Fig-1] [13]. The most optimum nutmeg toothpaste formula (Nutmeg Toothpaste 1-NT 1) was selected based on the results of foamability, pH, and spreadability testing against the standard Dabur red toothpaste [Table/Fig-2] [14,15]. Dabur red was chosen as the standard commercial herbal toothpaste (control toothpaste), based on an article published by the Government of India [14]. Other commercial herbal toothpastes were also considered, but various studies comparing Dabur red with other commercial toothpastes have found it to have superior qualities [14,16]. Hence, Dabur red was chosen as the standard control toothpaste for the present study.

Ingredients	Concentration (% w/w)		
	NT 1	NT 2	NT 3
Nutmeg powder	5	5	10
Tulsi leaves powder	0.8	0.8	1.6
Clove powder	0.2	0.2	0.4
Methyl paraben	0.2	0.2	0.4
Menthol	0.1	0.1	0.2
Titanium dioxide	0.4	0.4	0.8
Sodium lauryl sulphate	2.5	2	5
Honey	0.5	0.5	0.5
Water	7 mL	7 mL	15 mL

[Table/Fig-1]: Three different formulations of the nutmeg toothpaste.
(NT 1: Nutmeg Toothpaste1, NT 2: Nutmeg Toothpaste 2, NT 3: Nutmeg Toothpaste 3)

Criteria	Results			
	NT 1	NT 2	NT 3	Standard toothpaste (Dabur Red)
Foamability	5.8 cm	4.1 cm	4.3 cm	5.32 cm
Spreadability	Present	Present	Present	Present
		• Requires more pressure • Difficult to spread		
pH	5.85	6.01	6.09	4.68

[Table/Fig-2]: Results of the pilot study.
(NT 1: Nutmeg Toothpaste1, NT 2: Nutmeg Toothpaste 2, NT 3: Nutmeg Toothpaste 3)

The following ingredients were used to formulate the toothpaste under sterile conditions: nutmeg powder, Tulsi leaves powder, clove powder, methyl paraben, menthol, titanium dioxide, sodium lauryl sulfate, honey, and water q.s. [Table/Fig-3,4]. All herbal ingredients are 100% organic and certified by the Food Safety and Standard Authority of India (FSSAI). They were purchased commercially from the Green Pharmacy, Pune, and the chemical constituents were obtained from the AISSMS College of Pharmacy, Pune, Maharashtra, India.

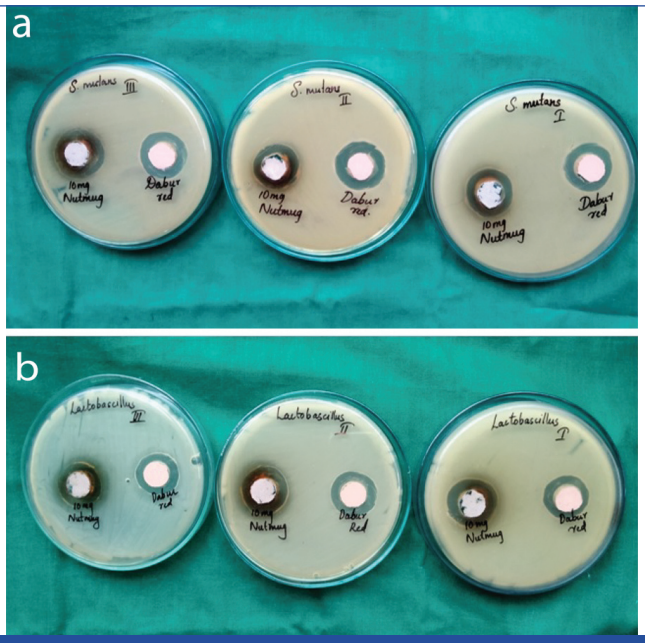
Constituent	Percentage (w/w)	Property
Nutmeg powder	28.41	Antibacterial and abrasive agent
Tulsi leaves powder	4.55	Prevents halitosis
Clove powder	1.14	Anti-inflammatory agent
Methyl paraben	1.14	Preservative
Menthol	5.68	Cooling agent
Titanium dioxide	2.27	Whitening agent
Sodium lauryl sulphate	14.20	Detergent
Honey	2.84	Humectant
Water q.s.	39.77	Vehicle

[Table/Fig-3]: Nutmeg toothpaste constituents.

[Table/Fig-4]: Toothpaste constituents being mixed together in a mortar and pestle.

The antimicrobial efficacy of the toothpaste was tested by evaluating the zone of inhibition against strains of *Streptococcus mutans* and *Lactobacillus* spp., which were collected from the National Collection of Industrial Microorganisms (NCIM), Pune. The microbial inhibition assay was performed using the agar well diffusion method. An adequate amount of Mueller Hinton Agar was evenly distributed over the surface of a petri dish and allowed to solidify under aseptic conditions. The strains of *Streptococcus mutans* and *Lactobacillus*

spp. were sub-cultured, and the turbidity of the culture solution was obtained at 0.5 McFarland Standard by adding the organisms. The test samples of both strains were obtained using the swab method and inoculated with a sterile spreader on the surface of solid Mueller Hinton Agar medium on different plates. Standard wells were made in the plates, and the test materials were inserted in the wells of different agar plates. These wells were filled with toothpastes, and the plates were incubated at 37±0.1°C for 24 hours. After incubation, the plates were observed for the zone of inhibition, and the diameters of these zones were measured in millimetres using vernier calipers. All the tests were performed following standard norms and protocols under sterile conditions [Table/Fig-5].



[Table/Fig-5a-b]: Evaluation of the zone of inhibition of the experimental and control toothpastes against *Streptococcus* and *Lactobacillus* spp.

The profilometric analysis was carried out on 24 permanent extracted human teeth that were collected, thoroughly cleaned of gross debris, and sectioned mesiodistally to produce flat labial surfaces. Enamel specimens of 5×5×5 mm were prepared and mounted on cold cure acrylic blocks [Table/Fig-6]. All 24 samples were then subjected to a profilometer to record the baseline values. A toothpaste slurry (toothpaste: water mixture) was prepared in a dilution of 1:3, and brushing was carried out twice daily for 28 days for a duration of two minutes using a standardised powered toothbrush and pea-sized amounts of both toothpastes. The specimens were stored in distilled water in between brushing, and profilometry was carried out on the 7th day and again on the 28th day of brushing. Changes in the surface roughness values as seen on the profilometer were recorded, calculated, and discussed [Table/Fig-7].

[Table/Fig-6]: Enamel specimens mounted on cold cure acrylic blocks, powered toothbrush and the experimental and control toothpastes.

[Table/Fig-7]: Profilometric analysis using a profilometer.

STATISTICAL ANALYSIS

The study data were analysed using Statistical Package for Social Sciences (SPSS) version 23.0 software, and appropriate statistical tests were applied. The comparison of the zone of inhibition of *Streptococcus mutans* and *Lactobacillus* spp. was done using the independent t-test. The repeated measures Analysis of Variance (ANOVA) test was used to compare the change in surface roughness within the experimental group and control group. Lastly, the intergroup comparison of surface roughness between the experimental and control group at each time interval was done using the independent t-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Antimicrobial testing: Both toothpastes exhibited antimicrobial activity against *Streptococcus mutans* [Table/Fig-8] and *Lactobacillus* spp. [Table/Fig-9]. However, the experimental toothpaste showed a significantly smaller zone of inhibition of 28 mm compared to the 32.17 mm observed with Dabur red against both microorganisms (p=0.003).

Groups	N	Mean	SD	Difference	p-value
Control	3	28.00	0.00	-4.17	**0.003***
Experimental	3	32.17	0.42		

[Table/Fig-8]: Comparison of the zone of inhibition for *Streptococcus mutans*. Independent t-test; *indicates a significant difference at p≤0.05

Groups	N	Mean	SD	Difference	p-value
Control	3	28.00	0.00	-4.17	**0.003***
Experimental	3	32.17	0.42		

[Table/Fig-9]: Comparison of the zone of inhibition for *Lactobacillus*. Independent t-test; *indicates a significant difference at p≤0.05

Profilometric analysis: The results of the comparison of changes in surface roughness revealed a significant increase in the enamel's surface roughness from the baseline to the 28th day for both toothpastes (0.43±0.17 to 0.57±0.25 for nutmeg toothpaste, p-value 0.037; and 0.65±0.20 to 0.81±0.20 for Dabur red, p-value 0.001). The values of the ANOVA test progressively increased until the 28th day [Table/Fig-10].

The intergroup comparison of surface roughness between different time intervals among the experimental and control groups revealed that the experimental (nutmeg) toothpaste showed a significant difference at baseline (p=0.010) [Table/Fig-11]. Therefore, to avoid

Groups	Baseline	7th day	28th day	p-value
Experimental	0.43±0.17	0.53±0.18	0.57±0.25	**0.037***
Control	0.65±0.20	0.75±0.20	0.81±0.20	**0.001***

[Table/Fig-10]: Comparison of change in surface roughness within the experimental and control groups.
Repeated measure ANOVA test; *indicates a significant difference at p≤0.05

bias, a comparison was made between the mean differences in surface roughness between the groups at day 7 and 28, which revealed that the difference was not statistically significant (p>0.05) [Table/Fig-12].

Interval	Groups	N	Mean	SD	Difference	p-value
Baseline	Experimental	12	0.43	0.17	0.22	**0.010***
	Control	12	0.65	0.20		
7th day	Experimental	12	0.53	0.18	0.22	**0.009***
	Control	12	0.75	0.20		
28th day	Experimental	12	0.57	0.25	0.24	**0.013***
	Control	12	0.81	0.20		

[Table/Fig-11]: Intergroup comparison of surface roughness between the experimental (nutmeg toothpaste) and control (Dabur red) groups at each time interval.
Independent t-test; *indicates a significant difference at p≤0.05

Interval	Experimental	Control	Difference	p-value
Baseline-7th day	0.09±0.03	0.10±0.02	-0.01	0.384
Baseline-28th day	0.13±0.17	0.17±0.03	-0.04	0.522
7th day-28th day	0.04±0.17	0.07±0.02	-0.03	0.617

[Table/Fig-12]: Evaluation of change in surface roughness between different time intervals among experimental (nutmeg toothpaste) and control (Dabur red) group, independent t-test.

DISCUSSION

In recent years, a paradigm shift has been observed in people's preference for herbal dentifrices over chemical ones. Herbal toothpastes have been formulated using a variety of ingredients, excluding *Myristica fragrans* or nutmeg. Limited studies have been conducted on the use of nutmeg (the seed kernels of *Myristica fragrans*) against oral microorganisms [2]. While nutmeg is mildly abrasive on the skin, there is insufficient research to conclude its efficacy on teeth. In the current study, Dabur red was selected as the standard commercial herbal toothpaste based on an article published by the Government of India. The article compared various commercially available herbal toothpastes and determined Dabur red to be the best based on sensory parameters and value for money [14].

Antimicrobial testing of the toothpaste was conducted against *Streptococcus mutans* and *Lactobacillus* spp., which are the two most common cariogenic organisms found in the oral cavity [17]. The agar well diffusion method using Mueller Hinton Agar was employed for this purpose [18-20]. Although the experimental nutmeg toothpaste exhibited significantly lower antimicrobial activity compared to the control [Table/Fig-1], it is evident that both toothpastes displayed some degree of antimicrobial activity against both microorganisms.

There is extensive evidence in the published literature supporting the antimicrobial efficacy of *Myristica fragrans*. Gupta A et al., reported that the acetone extract of *Myristica fragrans* exhibited antioxidant and antimicrobial activity, which could be attributed to compounds such as a-pinene, b-pinene, myrcene, 1,8-cineole, carvacrol, terpinen-4-ol, eugenol, and isoeugenol [21]. It was suggested that both minor and major compounds in *Myristica fragrans* contribute to its antimicrobial activity, with the possibility of minor compounds controlling the major ones [22]. Nikolic V et al., found that nutmeg essential oil demonstrated antimicrobial activity against various groups of microorganisms, including Gram-positive bacteria, Gram-negative bacteria, and fungi [23]. Shafiei Z et al., conducted two-fold serial microdilution tests and reported that the ethanol extract of nutmeg mace and seed exhibited good antibacterial activity against common oral pathogens [24]. Shetty JV et al., discovered that the essential oil of *Myristica fragrans* was effective against common endodontic microorganisms [25].

In the present study, the antimicrobial activity of *Myristica fragrans* is being tested as a part of a toothpaste. Therefore, the methodologies mentioned in the studies above are not directly applicable, as they only provide evidence supporting the antimicrobial activity of nutmeg. Previous usage of nutmeg in mouthwashes involved its essential oil form [26], which differs from the powder form used in the present study, thus altering its physical properties. Additionally, the abrasiveness of nutmeg would not be significant in its commercially available oil form. Hence, a toothpaste was formulated to evaluate the abrasiveness of nutmeg.

Another important property of a toothpaste is its abrasive action. In addition to reducing plaque and calculus build-up on teeth, the combination of toothbrush and dentifrice also aids in removing stains and discolorations [11]. Various types of abrasive agents present in toothpastes contribute to this action.

A certain level of abrasivity is necessary for a toothpaste to be effective. However, the uncontrolled use of abrasive agents can potentially have disastrous effects on tooth enamel and dentin, leading to hypersensitivity and recession [10]. This consideration is particularly important for deciduous dentition, as the enamel of primary teeth is thinner and can increase the risk of caries progression [11]. To address this, the International Standard Organisation (ISO) has set a maximum limit for Relative Dentine Abrasivity (RDA) at 250. The American Dental Association recommends an upper limit of 250, while the United States Food and Drug Administration suggests 200 [27]. Dentifrices with an RDA value over 100 are considered very abrasive and may cause harm to the enamel, dentin, and cementum. Low abrasive dentifrices typically have an RDA score below 70-80 [27].

The quantity of abrasive agent, particle size, surface structure, and chemical interactions with other components in a toothpaste all contribute to determining its abrasivity [28]. In the present study, a profilometer was used to assess the abrasivity of the toothpastes due to its accuracy compared to other methods, its non-damaging nature to the tooth surface during measurement, and the quick results it provides.

Based on the results of the profilometric analysis, both the experimental and control toothpastes significantly increased the surface roughness of the enamel from the baseline to the 28th day. Singh RP et al., reported that red-coloured dentifrices like Dabur red contain red ochre, which is responsible for their abrasive action [29]. Red ochre, primarily composed of hematite or dehydrated iron oxide, gives these dentifrices their reddish colour. Another study by Singla MG and Virdi I found that Dabur Red toothpaste exhibited significantly higher surface roughness compared to Vicco Vajradanti, Dantkanti, and Colgate Total, possibly due to the use of red-coloured dentifrices containing red ochre and iron oxide [28].

On the other hand, the abrasive action of the nutmeg toothpaste, attributed to nutmeg, could have also been influenced by the presence of other ingredients such as titanium dioxide [30]. Next, the evaluation of changes in surface roughness between different time intervals revealed that the nutmeg toothpaste showed lesser changes in surface roughness compared to Dabur red toothpaste, although the difference was not statistically significant. This indicates that the experimental toothpaste is comparable to the commercially available standard.

Since this is the first study of its kind regarding the abrasivity of nutmeg in a toothpaste format, there are currently no similar studies available in the published literature. The present study makes a significant contribution to the use of safer and natural ingredients in oral healthcare products.

Limitation(s)

This study had a few limitations, including the absence of the continuous washing action and the remineralisation effect of saliva on the teeth. Additionally, it did not incorporate an automated toothbrushing model and did not take into consideration the abrasive nature of toothbrushes. Furthermore, only one method was used to calculate the abrasivity of the toothpastes. Further in-vitro and in-vivo research, as well as studies on primary teeth, are warranted to test other significant properties of the nutmeg toothpaste.

CONCLUSION(S)

It can be concluded that the experimental nutmeg toothpaste and the control Dabur red toothpaste both show antimicrobial activity against *Streptococcus mutans* and *Lactobacillus* spp., although the experimental toothpaste showed significantly lesser activity. Additionally, both toothpastes significantly increased the surface roughness of the enamel from the baseline to the 28th day. The experimental nutmeg toothpaste caused less enamel abrasion than the control, but the difference was not statistically significant.

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