



Antibacterial Effect of Nutmeg Extract on Cariogenic Microflora in a Group of Egyptian Children

Rasha R. Ebrahim^{1*}, Mohammed H. Mostafa², Safaa R. El-Sayed³

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azhardentj@azhar.edu.eg

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ABSTRACT

Purpose: to evaluate the antibacterial effect of Nutmeg natural extract on cariogenic microflora and compare its effect with chlorhexidine (CHX) in a group of Egyptian children. **Subject and methods:** A total of 60 normal apparently healthy children aged between 6-12 years from both genders were involved in this study and equally divided into two groups (n=30) regarding to the type of the received mouthwash: group 1; included children who rinsed with 1% Nutmeg extract containing mouth wash (Tested group); group 2; included children who rinsed with 0.12% CHX containing mouth wash (Control group). Saliva samples were collected from each enrolled child at the base-line (before the use of mouthwash), and at 7th day after the use of mouthwash. Mitis salivarius bacitracin and Rogosa agar media were used to determine Streptococcus mutans (S. mutans) and Lactobacilli count respectively. **Results:** Significant reduction in S. mutans and Lactobacilli count in the two studied groups after mouth rinsing for 7 days. However, CHX showed a significant reduction in S. mutans and Lactobacilli count when compared to Nutmeg extract. **Conclusions:** Nutmeg extract has a significant positive antimicrobial effect against S. mutans and Lactobacilli microorganisms but the use of CHX mouthwash has a higher antimicrobial effect when compared to Nutmeg extract.

KEYWORDS

Cariogenic Microflora,
Egyptian Children,
Nutmeg Extracts

INTRODUCTION

Dental caries is one of the most prevalent chronic diseases among children and young adults' population ⁽¹⁾. Cariogenic microorganisms

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1. Dentist at Egyptian Ministry of Health, Egypt.
2. Associate Professor and Head of Pedodontics and Oral Dental Health, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt.
3. Lecturer of Pedodontics and Oral Dental Health, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt.

* Corresponding author email: k_morra22@yahoo.com

that colonize on tooth surface or present in the oral cavity such as *S. mutan* and *Lactobacilli* are considered risk factor for dental caries initiation and progression ^(2,3). Therefore, removal or at least control of dental plaque serve as the keystone for the proper oral hygiene practice ⁽⁴⁾.

The clinical evidence indicated that the mechanical measures for plaque control such as tooth brushing, and dental flossing cannot remove the colonized plaque completely ⁽⁵⁾. Therefore, effective mouth rinses can be used as adjunctive chemical approach for plaque control ⁽⁶⁾. Effective mouth rinses should have enough strength to change the oral microbiota via eliminating the harmful bacteria selectively without adversely affecting the commensals microorganisms ^(3,7).

Chlorhexidine is still the gold standard for mouth rinsing with the most effective antimicrobial properties but it has some shortcomings such as taste alteration, tooth and tongue staining, and increase the dental affinity for calculus formation ⁽⁸⁾. Thus, the need for synthesis and development of a new and safe antimicrobial mouth rinses were steadily endorsed especially in children ⁽⁹⁾.

Throughout the ages; nature has been a generous source for herbal products of effective antimicrobials potential against pathogenic bacterium because of their active chemical counterparts ^(3,10). Nutmeg is scientifically known as *Myristica fragrans* which has been broadly used as one of the national spices in Indonesian and parts of southern India and its flesh used extensively in production of sweets, syrup and essential oils ^(11,12).

Also, Nutmeg extracts is widely used in alternative medicine as it reported to have antiinflammatory, antidiarrheal, antimicrobial, and anti-cancer properties ⁽¹²⁾. The antimicrobial property of Nutmeg extracts may be attributed to the presence of active phytochemicals ingredients such as macelignan and trimyristin ⁽¹³⁾.

Nutmeg seed extracts were found to have macelignan, and trimyristin active compounds those exhibited a strong antimicrobial activity against Gram-positive and Gram-negative pathogenic bacteria ⁽¹²⁻¹⁴⁾. Therefore, this study was conducted to evaluate the antibacterial effect of Nutmeg natural extract on cariogenic microflora and compare its effect with CHX in a group of Egyptian children.

SUBJECT AND METHODS

This study was conducted on a total of 60 normal apparently healthy children with age range between 6-12 years from both genders from those attending outpatient's dental clinic of Pediatric Dentistry clinic at Faculty of Dentistry, Girl's branch, Al-Azhar University. Ethical approval was obtained from Ethical Committee of Faculty of Dental Medicine for Girls, Al-Azhar University (REC-PE-21-08). Written informed consent both written and verbal, was taken from the parents of each child before enrolling in the study.

Children selection was designed on the presence of low dmf for children and/or DMF for adult ("d/D"; decay, "m/M"; missing, "f/F"; filling) caries indices ≤ 4 , and the absence of any systemic disease, or orthodontic appliances, as well as no fluoride application or antibiotic and steroidal drug medication for at least 4 weeks before starting this study ^(15,16).

Sample size calculation:

Sample size calculation was based on a previous study by Varghese Suresh and Sapna⁽¹⁶⁾ to study the antibacterial effect of Nutmeg extract mouthwash on *S. mutan* and *Lactobacilli* in children. A sample size of 26 in each group was sufficient to detect a significance level for data.

Subject grouping:

Children were equally divided into two equal groups (n=30) according to the type of the received mouth rinse: group 1; included children who rinsed

with 1% Nutmeg extract containing mouth wash (Tested group); group 2; included children who rinsed with 0.12% CHX containing mouth wash (Control group).

Preparation of 1% Nutmeg mouth-rinse:

A total of 20 g of nutmeg flesh were soaked in 100 ml of hot sterile water and allowed to stand for 72 hrs. The extract was considered as 100% in concentration. The crude extracts were filtered. The concentrations 1% were made by diluting the concentrated extract with the required volume of sterile distilled water ⁽¹⁷⁾ (Figure 1).

Patient instructions:

Each enrolled child was instructed to do regular tooth brushing using modified Bass brushing technique. Children were instructed to brush twice daily (in the morning and evening) for two weeks. Then, the enrolled children were instructed to rinse their mouth with 10 ml of the solution for 2min twice daily after tooth brushing followed by expectoration of the residual mouth rinse ^(18,19). Tooth brushing and mouth rinsing techniques were demonstrated for every child.

After mouth rinsing, the subjects were adjusted not to eat or rinse for the next 30 minutes and frequent reminders through telephone were given to supervisors (parent's/caregivers) to insure

compliance and not to take any antibiotics without reference to operator.

Saliva sample collection:

During the study, two saliva samples were collected from each enrolled child; before the use of mouthwash (base-line), and after 7th day of mouth rinsing. Before collection of each saliva sample, each child was asked to rinse mouth with deionized water for 1 minute before sampling ⁽¹⁸⁾.

Samples were delivered as soon as possible to the microbiological lab at Microbiology and immunology Department, Faculty of Medicine for Girl's, Al-Azhar University for culturing on freshly prepared selective media of *Mitis salivarius* bacitracin and Rogosa agar media to determine *S. mutans* and *Lactobacilli* count respectively ^(15,20).

Statistical analysis

The collected data were tabulated and statistically analyzed using SPSS program software, version 20. The independent t-test and Chi-Square test were applied to gauge the difference between demographic data. Paired t-test and independent t-test test were used to compare between sample means for quantitative data with normal distribution. The results were considered statistically significant at $p \leq 0.05$.

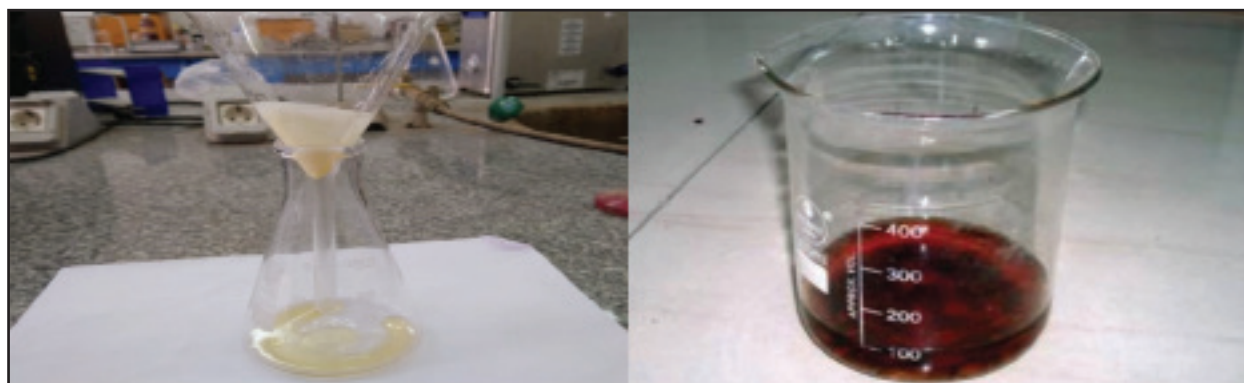


Figure (1): Nutmeg extraction.

RESULTS

Demographic background:

The statistical analysis of age for the two studied groups revealed that; there was no-statistically significant difference as indicated by independent t- test, the P-value equals ($P= 0.221$), between the recorded mean age values among the two studied groups Table (1). While, the statistical analysis of gender for the two studied groups revealed that; there was statistically significant difference as indicated by chi-square test, the p-value equals ($P= 0.019$), between the recorded variables among the

two studied groups (Figure 2 and 3).

Microbial count:

The statistical results showed that *S. mutans* and Lactobacilli count recorded a significant reduction in the both studied groups after rinsing for 7 days. However, regarding to the recorded *S. mutans* and Lactobacilli count at base-line (before mouth rinsing) there was no significant differences were found among the two studied groups. while the CHX group recorded the lowest significant bacterial count when compared with Nutmeg group in regard to *S. mutans* and Lactobacilli count (Figure 4).

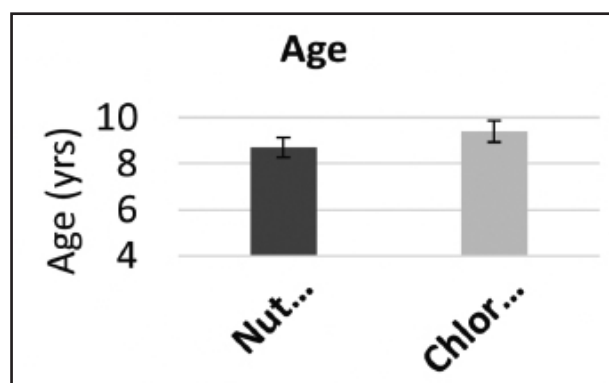


Figure (2) Age distribution along the study.

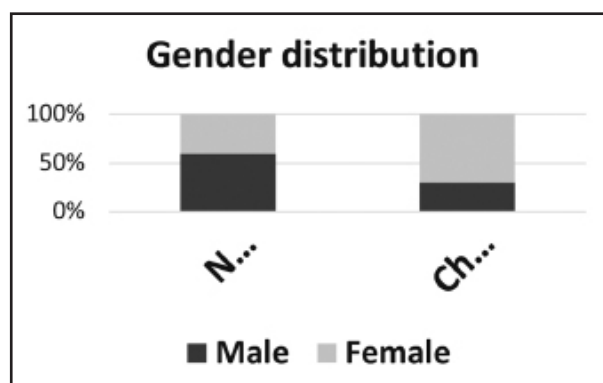


Figure (3) Gender distribution along the study.

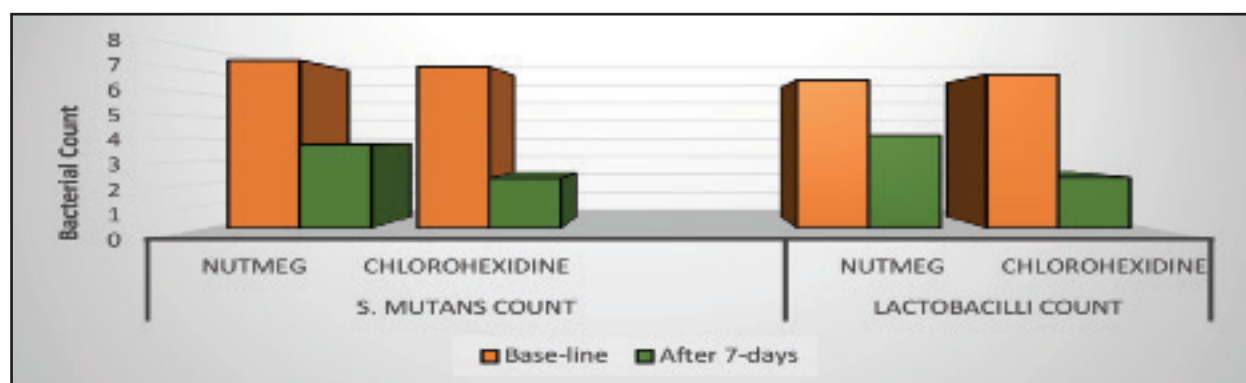


Figure (4): Bacterial count along the study.

DISCUSSION

Caries is a chronic infectious disease that follow bacterial colonization on hard tooth structures and although the global efforts that exerted to decrease its incidence, its prevalence is still high^(1,3). Chemical antibacterial agents including mouth rinses are considered an important tool to control and/or reduce the bacterial colonization along with the usual mechanical methods⁽⁷⁾.

In the present study, the enrolled children were aged from 6-12 years as they can easily rinse their mouths without swallowing the rinsing solutions to avoid deglutition reflex's⁽²⁰⁾.

In the current study, *S. mutans* and *lactobacilli* were chosen as a tested pathogenic microorganism for comparison of the antibacterial activity of Nutmeg and CHX mouth rinses. This is because *S. mutans* is the bacterial species that responsible for the initiation of caries while *lactobacilli* are the responsible for caries progression⁽¹⁶⁾. The Mitis salivarius bacitracin and Rogosa agar media were chosen in the current study for isolation, colonization, and counting of *S. mutans* and *Lactobacilli* respectively because they are the selective media for such colony's growth and inhibition of other microorganisms⁽²¹⁾.

A 0.12% chlorhexidine mouth rinse was used as an active control in the current study. As CHX is the most popular and the most effective antimicrobial agent until now, as well as it is marketed as the gold standard among all other mouthwashes^(16,18). However, due to its known shortcoming, there is a continuous need to formulate new safer mouthwashes specifically for children^(8,16,19). Therefore, herbal extracts with their active phytochemical ingredients offer the least harmful, and the most truthful alternative way for the rehabilitation of the oral health^(3,10).

Nutmeg extracts are extensively used in unconventional medicine and it recorded to have antimicrobial effect^(13,16). Their antimicrobial action mode is pertinent to their capacity to inactivate microbial enzymes, cell enveloping proteins, and prevent bac-

terial adhesion⁽¹³⁾. Thus, Nutmeg extract should be considered having beneficial potential in dentistry as mouthwash.

The nutmeg extract was prepared by the investigator at 1% concentration with the reference from previous studies which showed the maximum zone of inhibition⁽¹⁶⁾. Furthermore, it was found that 0.12% chlorhexidine mouthwash when used as a mouth wash was found to be effective in reducing the salivary *S. mutans* count^(8,16,18).

This study detected the number *S. mutans* and *Lactobacilli* oral pathogens prior to the use of the tested mouth rinses with no statistically significant differences in tested groups ($p = 0.21$, and 0.31), so this referred to standardization for bacterial counts in the both studied groups.

According to the results of the present study it was found that 1% Nutmeg mouthwash was effective in significantly reducing the salivary *S. mutans* and *Lactobacilli* count ($P=0.00$). This may be because of the Nutmeg contains the myristic acid and trimyristin which are antibacterial compounds that extracted from Nutmeg⁽¹²⁾. Furthermore, the mode of antimicrobial action of Nutmeg extraction is related to their ability to inactivate microbial enzymes, and cell envelope proteins⁽¹⁶⁾.

In this study it was found that 0.12% CHX mouthwash was significantly effective in reducing the salivary *S. mutans* and *Lactobacilli* count ($P=0.00$). This may be because of the fact that CHX has the ability to increases the permeability of cell membrane followed by coagulation of cellular macromolecules. Moreover, it does not interact with any microbial enzymes or receptors and hence does not lead to bacterial resistance^(16,20).

CONCLUSION

Based on the finding of the current study it could imply that 1% Nutmeg mouth wash has an antimicrobial effect however, CHX has the marked antibacterial efficiency regarding *S. mutans* and *lactobacilli* species.

RECOMMENDATIONS

- The use of herbal products should be investigated and considered as an alternative effective mouthwash.
- Nutmeg extracts should be investigated as alternative mouthwash at different concentration.
- Different extract methods of Nutmeg should be investigated in the future studies.

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CONFLICT OF INTEREST

No conflict of interest.

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