ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 4, Issue – 2, March - 2021, Page No. : 460 - 469

Efficacy of Myristica fragrans extract in root canal irrigation of primary teeth - A clinical trial

[1]Dr. Jyothsna V Setty, Professor, Department of Pedodontics and Preventive Dentistry, M. R. Ambedkar Dental College and Hospital, Bengaluru.

[2]Dr. Ila Srinivasan, Professor and Head, Department of Pedodontics and Preventive Dentistry, M.R.Ambedkar Dental College and Hospital, Bengaluru.

[3]Dr. Roopashree T.S, Head, Department of Pharmacognosy, Government College of Pharmacy, Bengaluru.

[4]Dr. Mamata Kale, Associate Professor, Department of Microbiology, B. R. Ambedkar Medical College and Hospital, Bengaluru.

[5]Dr. Swathi Kakathkar, Post Graduate Student, Department of Pedodontics and Preventive Dentistry, M.R. Ambedkar Dental College and Hospital, Bengaluru, Karnataka, India-560005

[6]Dr Akhil P, Post Graduate Student, Department of Pedodontics and Preventive Dentistry, M.R.Ambedkar Dental College and Hospital, Bengaluru.

Corresponding Author: Dr. Jyothsna V Setty, Professor, Department of Pedodontics and Preventive Dentistry, M. R. Ambedkar Dental College and Hospital, Bengaluru.

Citation of this Article: Dr. Jyothsna V Setty, Dr. Ila Srinivasan, Dr. Roopashree T.S, Dr. Mamata Kale, Dr. Swathi Kakathkar, Dr Akhil P,"SEM Evaluation of effect of IgY based oral passive immunization against dental caries- An Invitro study", IJDSIR- March - 2021, Vol. – 4, Issue - 2, P. No. 460 – 469.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Aim: To evaluate the antimicrobial efficacy of Myristica fragrans, sodium hypochlorite (1%) and chlorhexidine (2%) and normal saline (control) in root canal disinfection of primary teeth**.**

Methods: A total of 60 children aged 4-8 years who required pulpectomy were divided into four groups of 15 each. In Group A 1 % sodium hypochlorite was used as an irrigant, in Group B 2% chlorhexidine was used as an irrigant and in Group C Myristica fragrans extract was used. Group D was control group where 0.9 % normal saline was used as an irrigant. Pre and post-operative samples were collected using absorbent paper points and subjected to microbiological culture. Total colony forming units (CFU s) of both aerobic and anaerobic organisms were counted at both the periods. Total reduction in number of CFU s in all the groups was counted and statistically analysed.

Results: All the four groups showed a significant decrease in bacterial load at the post-operative period with 2 % chlorehexidine showing a maximum antimicrobial

efficacy followed by Myristica fragrans extract then 1 % sodium hypochlorite and lastly normal saline.

Conclusion: Myristica fragrans extract can be used as promising alternative in root canal disinfection of primary teeth.

Keywords: Canal disinfection, Chlorhexidine, Myristica fragrans, Primary teeth, sodium hypochlorite.

Introduction

Primary tooth is always the best space maintainer in both primary and mixed dentition .This is not only because of the clinical crown but also due to the presence of roots and periodontium that guide the eruption of succedaneous permanent teeth. Primary teeth also stimulate development of maxilla and help in masticatory process thus preserving the integrity of primary dentition is important for the development of permanent dentition.[1]

The ultimate aim of endodontic therapy is to eliminate the pathogenic flora from the root canals and maintain a sterile environment before obturation. Endodontic treatment in paediatric patients is challenging because of variations in root anatomy, root resorption, presence of accessory canals along with difficulty faced in achieving patient cooperation.

The use of files and irrigating solutions with disinfectant properties is currently the most common form of endodontic treatment. The existence of residual bacteria in many cases can lead to further infection of the root canal, which requires re-treatment or even extraction.[2]Sodium hypochlorite (NaOCl) appears to be the most commonly used irrigant in a concentration ranging from 0.5 to 6% (Walker, 1936). The factors contributing to its popularity are low cost, ease of availability and good shelf life, potential bactericidal activity and excellent tissue dissolving action. Chlorhexidine gluconate is another irrigant widely used in dentistry which is having antiseptic and bactericidal properties. One of the most important properties of chlorhexidine(CHX) is substantively contributing to its prolonged antimicrobial activity (Zehnder, 2006)[3]. However, these chemical agents have various disadvantages.

Sodium hypochlorite though considered an ideal root canal irrigant, cannot be used at higher concentrations in children due to its detrimental effects or limitations, such as unpleasant taste, odour, toxicity and ineffectiveness in removing smear layer. Periapical extrusion of NaOCl causes immediate pain, edema of neighbouring soft tissues, ecchymosis, and paresthesia, due to tissue response. Similarly, chlorhexidine digluconate, although widely used in disinfection because of its excellent antimicrobial activity, completely lacks tissue dissolving capability.[4]

To overcome the short comings of current materials, a shift towards mother nature with various herbal agents are being studied. Nutmeg, Myristica fragrans is one such nature's gift, originally used as a spice has been investigated for a variety of biological properties. The constituents of Myristica fragrans are found to have antimicrobial, anti-inflammatory, antioxidant, antidiabetic, antidepressent, memory enhancing, hepatoprotective, pesticidal and anticancerous activity.[5] Myristica fragrans extract is also shown to be effective on most of the endodontic pathogens.[6]

Therefore, the aim of this study is to evaluate the antimicrobial efficacy of Myristica fragrans extract and to compare it with sodium hypochlorite (1%) and chlorhexidine (2%) in root canal disinfection of primary teeth.

Methodology

The study was conducted in the Department of Pedodontics and Preventive Dentistry, M.R. Ambedkar Dental College and Hospital, Bengaluru, Karnataka. The samples were selected from patients coming to the

department based on inclusion and exclusion criteria. The study was commenced after obtaining approval from the Institutional Ethical Committee and Review Board. Based on published literature, the sample size for the present study was estimated using the software G Power v. 3.1.9.2 .Considering the effect size to be measured (f) at 40%, power of the study at 85% and the margin of error at 5%, the total sample size needed was 60.Thus, each group comprised of 15 samples [15 subjects x 4 groups = 60 samples].

Inclusion criteria

Patients who belonged to Frankel's 3 and Frankel's 4 behavioural rating scale, between the age of 4-8 years of both the sexes with no history of systemic illness and who were not under any medication.

1. Teeth with carious pulp exposure which were indicated for pulpectomy.
2. Teeth with at least 2/3rd of intact root length.
3. Teeth which were restorable.
4. Patients who willingly gave the assent and for whom the parents or the legal guardian gave the written informed consent to be a part of this study.
5. Exclusion criteria:
6. Teeth with preshedding or abnormal pathologic mobility.
7. Teeth with root resorption more than 1/3rd of the root length.
8. Teeth which showed radiographic signs of inter-radicular involvement ranging from slight thinning of the trabeculae to furcal radiolucency.
9. Non restorable teeth.
10. Teeth which showed perforation of the pulpal floor.
11. Patients with special health care needs.
12. Patients who were not willing or for whom the parents or the legal guardian did not give the written informed consent for the treatment.

Patients who were suffering from any other debilitating systemic illness.

Selected sample was randomly allocated to any of the 4 groups by using chit picking method until the sample size of 15 in each group was reached. Pre-operative radiographs were taken to assess the extension of the carious lesion. Before beginning the procedure, all the instruments were autoclaved and non- autoclavable instruments were chemically disinfected. Administration of local anesthesia (2% lignocaine hydrochloride) and isolation of tooth using rubber dam was done. Straight line access cavity preparation was done using a high speed airotor hand piece. Determination of working length was done using Ingle's method. Collection of first sample (S1) was done by introducing the sterile paper points with anatomic diameter compatible to the root canal for 30 seconds, which was transferred to the Eppendorf vial containing normal saline and stored in the refrigerator until sent for microbiological assessment.[7, 8] Biomechanical preparation (BMP) was performed in the same appointment using 1% NaOCl as an irrigant in group A.(Vensons India, Sampige road, Bangalore.).[9] using 2% chlorhexidine as an irrigant in group B. (Stedman pharmaceuticals private Ltd. Alathur, Thiruporur, Tamilnadu). In group C Myristica fragrans (standardized) was used for irrigation. Group D was used as a control group where normal saline was used for canal irrigation. Commercially available 3% NaOCl solution was diluted (1:3) to 1% concentration using normal saline.[10] Following this, collection of second sample (S2) was done by introducing sterile paper points with anatomic diameter compatible to the canal for 30 seconds, which was transferred to the eppendorf vial containing normal saline and was sent immediately for microbiological assessment along with first sample (S1)[7,8] Prepared tooth was

obturated in the consequent appointment followed by final restoration.

The vials containing the samples in normal saline were thawed at 36°C in a water bath and vortexed for 30–45 seconds. Undiluted sample (200-300µl) was serial diluted and serial dilutions (10^{-1} to 10^{-3}) were plated on blood agar plates (HiMedia Lab Pvt. Ltd., India), by spread plate technique. Blood agar plates were incubated at 37°C and 5%–10% CO_2 atmosphere for 5 days to cultivate aerobic and facultative anaerobic bacteria. Serial dilutions of sample were plated on blood agar plates by pour plate technique. Blood agar plates were incubated anaerobically in anaerobic chamber at 37°C for 5-10 days. Total count was determined and expressed as colony forming unit (CFU) per ml of samples.Total viable bacterial count before and after biomechanical preparation and disinfection in different groups was determined. The values were tabulated and subjected for statistical analysis.

Statistical analysis

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp. was used to perform statistical analyses. Descriptive analysis of all the explanatory and outcome parameters was done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables. Kruskal Wallis Test followed by Mann Whitney post hoc Analysis was used to compare the Mean viable bacterial count before and after root canal disinfection procedure. Wilcoxon Signed Rank Test was used to compare the Mean viable bacterial count between before and after root canal disinfection procedure in each study group. The level of significance was set at P<0.05.

Results

Comparison of mean difference in CFUs of aerobic and anaerobic organisms between different groups during post-operative period showed a statistically significant difference among all the groups with 2% chlorhexidine showing the highest antimicrobial efficacy followed by Myristica fragrans extract, 1% NaOCl and normal saline in decreasing order.(Table 1,Table 2,Figure 1,Figure 2,Figure 3,Figure 4)

When multiple comparison of mean between the groups was made, statistically significant reduction in both aerobic and anaerobic CFUs was seen.(Table 3,Table 4)

Groups	N	Mean	SD	Min	Max	P-Value
1% NaOCl	25	101.76	84.46	0	300	<0.001*
2% CHX	25	1.76	1.99	0	6	
Nutmeg Extract	25	23.08	18.08	1	55	
Saline	25	154.68	111.30	12	432	

Table 1: Comparison of mean CFUs [x 10^2 / ml] of Aerobic Microbes during Post-operative period using Kruskal Wallis Test

Groups	N	Mean	SD	Min	Max	P-Value
1% NaOCl	25	112.24	115.36	0	320	<0.001*
2% CHX	25	0.16	0.55	0	2	
Nutmeg Extract	25	39.88	38.06	0	150	
Saline	25	190.60	159.32	10	487	

Table 2: Comparison of mean CFUs [x 10^2 / ml] of Anaerobic Microbes during Post Operative period using Kruskal Wallis Test

(I) Groups	(J) Groups	Mean Diff.(I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
1% NaOCl	2% CHX	100.00	47.90	152.10	<0.001*
	Nutmeg Extract	78.68	26.58	130.78	<0.001*
	Saline	-52.92	-105.02	-0.82	0.04*
2% CHX	Nutmeg Extract	-21.32	-73.42	30.78	<0.001*
	Saline	-152.92	-205.02	-100.82	<0.001*
Nutmeg Extract	Saline	-131.60	-183.70	-79.50	<0.001*

Table 3: Multiple comparison of mean difference in CFUs [x 10^2 / ml] of Aerobic Microbes during Post-operative period using Mann Whitney Post hoc Test

(I) Groups	(J) Groups	Mean Diff.(I-J)	95% CI for the Diff.		P-Value
			Lower	Upper	
1% NaOCl	2% CHX	112.08	38.00	186.16	<0.001*
	Nutmeg Extract	72.36	-1.72	146.44	0.08
	Saline	-78.36	-152.44	-4.28	0.03*
2% CHX	Nutmeg Extract	-39.72	-113.80	34.36	<0.001*
	Saline	-190.44	-264.52	-116.36	<0.001*
Nutmeg Extract	Saline	-150.72	-224.80	-76.64	<0.001*

Table 4: Multiple comparison of mean difference in CFUs [x 10^2 / ml] of Anaerobic Microbes during Post operative period using Mann Whitney Post hoc Test

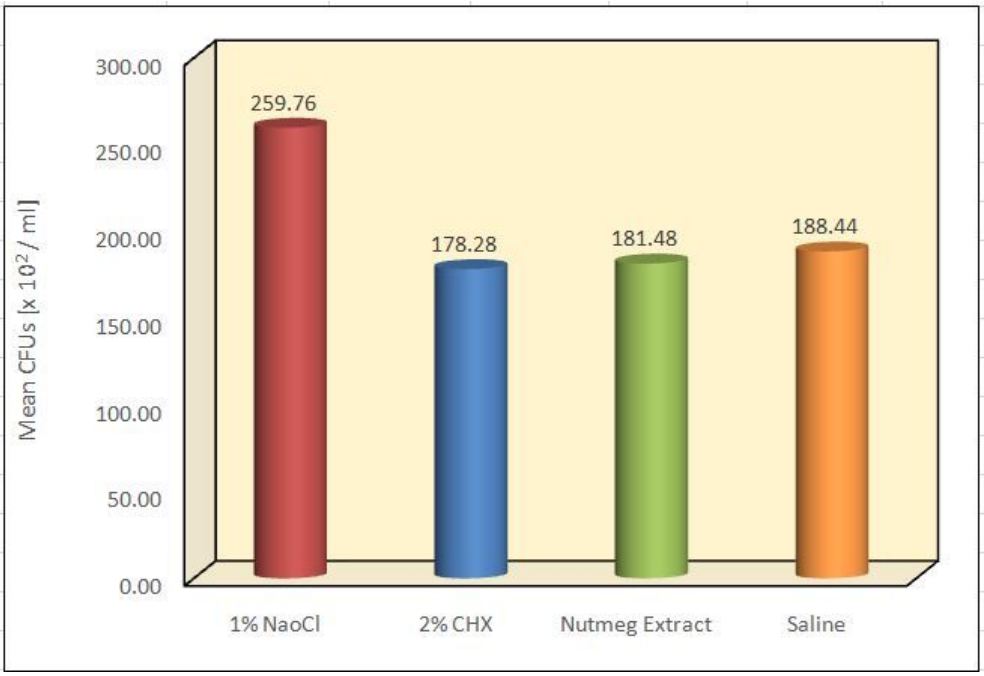

Figure 1: Mean CFUs [x 10^2 / ml] of Aerobic Microbes during Pre operative period

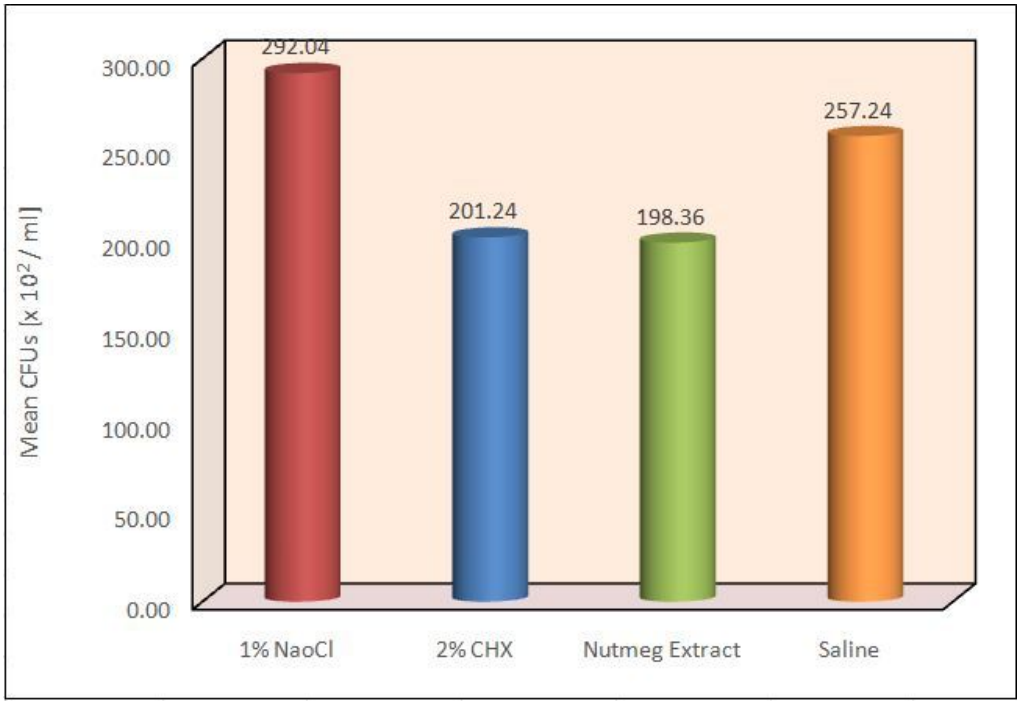

Figure 2: Mean CFUs [x 10^2 / ml] of Anaerobic Microbes during Pre operative period

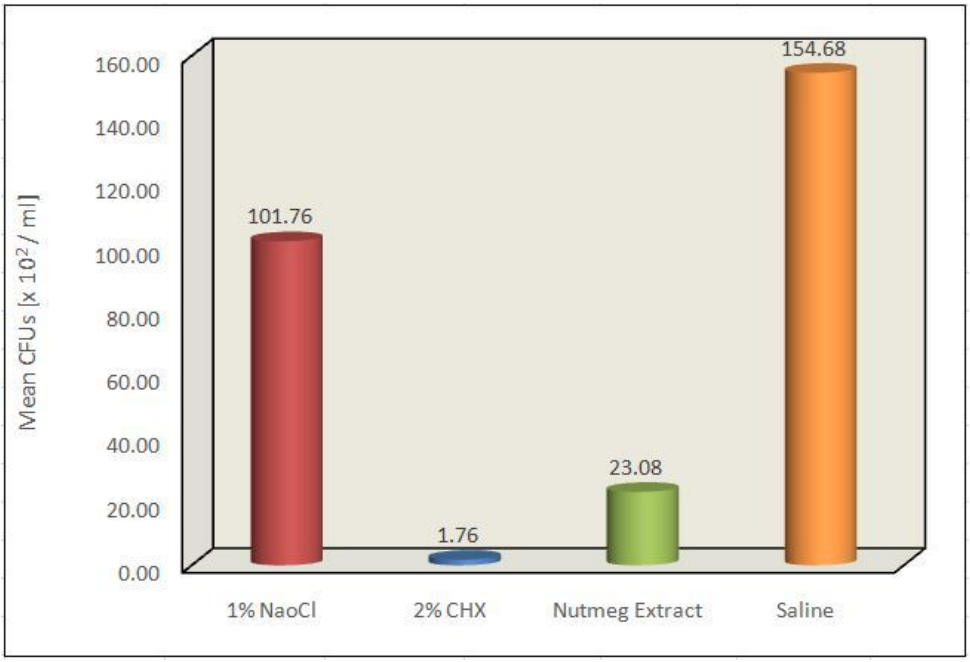

Figure 3: Mean CFUs [x 10^2 / ml] of Aerobic Microbes during Post operative period

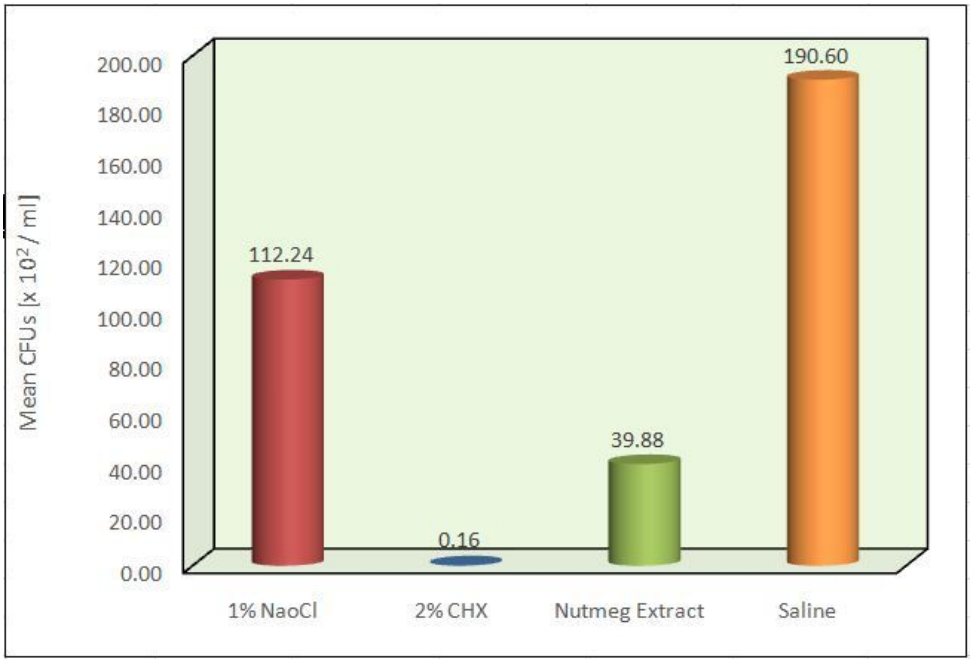

Figure 4: Mean CFUs [x 10^2 / ml] of Anaerobic Microbes during Post operative period

Discussion

The main objective of root canal treatment is complete elimination of microorganisms from the infected root canals. Although cleaning and shaping reduce microorganisms, the use of irrigants is complimentary to instrumentation in facilitating their removal. Several chemicals and therapeutic agents are used to achieve this goal among which NaOCl and chlorhexidine are widely used. The gold standard and the most effective among these is sodium hypochlorite (NaOCl). However, owing to the potential side effects, safety concerns, and cytotoxic reactions of synthetic irrigants, usage of various herbal agents has increased over the last few decades, and the search is on for a root canal irrigant that can match or

better the gold standard. Although chlorhexidine does not have many disadvantages as sodium hypochlorite, there are studies showing cytotoxic effects of chlorhexidine and even it does not taste good for use amongst children and also causes teeth discoloration. Herbal products have been used since ancient times in dental and medical practice, and the trend is growing now due to their high antimicrobial activity, excellent biocompatibility, anti inflammatory, and antioxidant properties.[11, 12]

The ideal properties of a root canal irrigant are: it should be systemically nontoxic, should not harm the periodontal tissues, should not cause an allergic reaction, should possess a broad antimicrobial spectrum, should be capable of dissolving necrotic pulp tissue, inactivating endotoxins, and either preventing the formation of a smear layer or dissolving it once it has formed. Herbal or natural products have also become more popular today due to their high antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties. A wide variety herbal products have been used in the past in medicine such as propolis, neem, aloe vera, morinda, triphala, green tea extract and many more[13]

M. fragrans extracts have shown to be very effective against oral microorganisms such as S. mutans. Essential oil of M. fragrans has excellent antimicrobial activity against endodontic pathogens of primary teeth as shown by its minimum inhibitory activity. Hence, M. fragrans can be considered in various formulations used for the treatment of infectious pulpal conditions. A study done by Vinothkumar et al.[14] found M. fragrans extract to be effective against E. faecalis and C. albicans when used as an endodontic irrigant. Considering these beneficial effects, especially antinflammatory and antibacterial activities, the present study was intended to investigate the effect of M. fragrans against standard irrigating solutions.[6]

Essential oils obtained from Myristica fragrans seeds have growth inhibition capability against bacterial spores and can act as food preservatives. It has been observed experimentally that extract from the dried seed cover of Myristica fragrans contains two compounds and both exhibit strong antifungal and antibacterial activities. In another study, it was found that ethyl acetate extract of the fresh Myristica fragrans had strong bactericidal activity against some cariogenic Gram-positive and Gram-negative bacteria. The extract from seeds of Myristica fragrans used for the treatment of inflammatory diseases also had inhibitory effects on nitric oxide (NO) production.[15]

A culture dependent approach was used in present study, as it is one of the most reliable methods of detecting viable bacteria, particularly when samples were taken immediately after antimicrobial treatment where viability may not be ascertained by most culture-independent methods.[16] The aerobic and anaerobic colonies were counted manually to get the total CFU's .Similar microbiological protocol was also used in the study conducted by Prabhakar et al[7].

In the present study we found that M. fragrans extract significantly reduced the intra canal bacterial load post operatively and was found to be better than 1% NaOCl but not as effective as 2% chlorhexidine. Both aerobic and anaerobic CFU count showed a significant decrease in the post-operative period.(Table 1,Table 2) Similar results were obtained in a study conducted by Mali et al.[17], to evaluate and compare the effectiveness of Myristica fragrans, Myrobolan, Tulsi, and 2.5% sodium hypochlorite (NaOCl) on the removal of the smear layer by the scanning electron microscope (SEM). The results showed that all herbal extracts were found to be significantly effective than 2.5% NaOCl. In an in vitro study conducted by Vinothkumar et al. , five different herbal extracts were

evaluated as anti-microbial endodontic irrigants. The efficiency of the extracts in descending order was as follows: A. indica, C. Longa, Myristica fragrans, Terminalia chebula, and Aloe barbadensis which support the results of the present study. [14]

So, the major advantages of herbal irrigants are safety, easy availability, increased shelf life, cost effectiveness and lack of microbial resistance .The studies conducted till date have shown that herbal products can have a promising role as canal irrigants. This study has brought into light the antimicrobial efficacy of M. fragrans in primary teeth against endodontic bacterial load. However, further clinical trials and investigations are required to be considered for M. fragrans to be considered as an effective alternative to the conventional canal irrigants.[13]

Conclusion

From this study the following conclusions can be drawn:

Among the root canal irrigants tested, 2% chlorhexidine showed maximum disinfecting properties followed by Nutmeg on both aerobic and anaerobic microorganisms. Myristica fragrans extract showed better disinfecting properties when compared with 1% NaOCl and normal saline. Hence, Myristica fragrans extract can be used as promising alternative in root canal disinfection of primary teeth.

Acknowledgement: The authors would like to thank and acknowledge Dr. Santhosh for helping with statistical analyses and Dr.Chandrashekhar B.R, Professor and Head, Department of Microbiology, Dr B.R.Ambedkar Medical college and Hospital, Bangalore for guiding and helping with the microbiological procedures.

References

1. Brothwell DJ. Guidelines on the use of space maintainers following premature loss of primary teeth. J Can Dent Assoc 1997;63(10):753-66.
2. da Mota AC, Gonçalves ML, Bortoletto C, Olivan SR, Salgueiro M, Godoy C, Altavista OM, Pinto MM, Horliana AC, Motta LJ, Bussadori SK. Evaluation of the effectiveness of photodynamic therapy for the endodontic treatment of primary teeth: study protocol for a randomized controlled clinical trial. Trials 2015 ;16(1):551.
3. Jose J, Shoba K, Aman S, Tomy N. Microbiological Evaluation of the Level of Disinfection Attained by Mtad, 2% Chlorhexidine and 2.5% Sodium Hypochlorite A Comparative in vivo Study. Int J Curr Res Acad Rev 2015;3(10):157-63.
4. Agarwal S, Tyagi P, Deshpande A, Yadav S, Jain V, Rana KS. Comparison of antimicrobial efficacy of aqueous ozone, green tea**,** and normal saline as irrigants in pulpectomy procedures of primary teeth. J Indian Soc Pedod Prev Dent 2020;38:164-70.
5. Preetee J, Kumar P, Singh VK, Singh DK. Biological effects of Myristica fragrans. ARBS Annu Rev Biomed Sci 2009;11:21-9.
6. Setty JV, Srinivasan I, Ssthiesh RT, Kale M, Shetty VV, Venkatesh S. In vitro evaluation of antimicrobial effect of Myristica fragrans on common endodontic pathogens. J Indian Soc Pedod Prev Dent 2020;38:145-51.
7. Prabhakar AR, Yavagal C, Agarwal S, Basappa N. Antimicrobial Effects of Laser-assisted Photodynamic Therapy in Pediatric Endodontic Treatment: A New Clinical Horizon. Int J Laser Dent 2013;3(3):77-81.
8. Thomas S, Asokan S, John B, Priya G, Kumar S. Comparison of Antimicrobial Efficacy of Diode Laser, Triphala, and Sodium Hypochlorite in Primary Root Canals: A Randomized Controlled Trial. Int J Clin Pediatr Dent 2017;10(1):14-7.
9. Kaur R, Singh R, Sethi K, Garg S, Miglani S. IrrigatingSolutions in Pediatric Dentistry: Literature

Review and Update. J Adv Med Dent Scie 2014;2(2):104-115.

10. Singhal P, Das UM, Vishwanathan D, Singhal A. Carisolv as an endodontic irrigant in deciduous teeth: An SEM study. Indian J Dent Res 2012;23:120-1.
11. Gupta P, Kulkarni S, Gajeshwar H. Comparative evaluation of antibacterial efficacy of chlorhexidine gluconate, fenugreek (Trigonella foenum) and Fennel (Foeniculum vulgare) as intracanal irrigant on isolated bacteria from infected primary tooth. IJAR. 2018;4(2):162-5.
12. Podar R, Kulkarni GP, Dadu SS, Singh S,Singh SH. In vivo antimicrobial efficacy of 6% Morinda citrifolia, Azadirachta indica, and 3% sodium hypochlorite as root canal irrigants. Eur J Dent 2015;9:529-34.
13. Jain P, Ranjan M. Role of herbs in root canal irrigation-A review. IOSR J Pharm Biol Sci. 2014;9(2):06-10.
14. Vinothkumar TS, Rubin MI, Balaji L, Kandaswamy D. In vitro evaluation of five different herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. J Conserv Dent. 2013 Mar;16(2):167-70.
15. Mali S, Singla S, Sharma A, Gautam A, Niranjan B, Jain S. Efficacy of Myristica fragrans and Terminalia chebula as Pulpotomy Agents in Primary Teeth: A Clinical Study. Int J Clin Pediatr Dent, 2018;11(6):505-509
16. Sohrabi K, Sooratgar A, Zolfagharnasab K, Kharazifard MJ, Afkhami F. Antibacterial activity of diode laser and sodium hypochlorite in enterococcus faecalis-contaminated root canals. Iran Endod J. 2016;11(1):8.
17. Mali S, Singla S, Tyagi P, Sharma A, Talreja N, Gautam A. Comparative evaluation of the efficacy of different herbal irrigants on the removal of smear layer of primary teeth: A scanning electron microscopy study. J Indian Soc Pedod Prev Dent 2020;38:374-80