"Comparison of the antimicrobial efficacy of combinations of nutmeg, aloe vera and neem as an intracanal medicament with calcium hydroxide on enterococcus faecalis – an *invitro* study."

ABSTRACT:

BACKGROUND:Intracanal medicament should have antibacterial effect on the infected root canal bacteria for the success of endodontic procedures. Calcium hydroxide is currently the 'golden standard' intracanal medicament but it still has disadvantages like internal roor resorption. The search for an effective and ideal intracanal medicament is an ongoing process that is still elusive. In an attempt to search for new materials in future, we, in this pilot study are trying to draw inspiration from the past. Ayurveda, originated in India more than 3000 years ago. There is a renewed interest in herbal products in recent years as many have demonstrated antibacterial, antiseptic, and analgesic properties.

AIM: To compare in-vitro, the antibacterial efficacy of a combination of herbal extracts i.e nutmeg + aloe vera, nutmeg + neem and nutmeg + distilled water with calcium hydroxide as an intracanal medicament on *Enterococcus faecalis.*

MATERIALS AND METHOD: The study included*E. Faecalis* bacterial ATCC strain – 29212. AMueller Hinton agar plate was inoculated with *E. faecalis.* Four wells with diameter 8-10mm were punched and the Herbal extract combinations were pipetted into the agar wells. Calcium hydroxide, which is the golden standard in intracanal medicament, was used as a control after which, the plate is incubated. The diameter of the inhibition zones around all the wells were measured manually after incubation for 48hrs.

RESULTS: Highest zone of inhibition was seen around the wells containing Nutmeg and its combinations. Nutmeg showed better antibacterial efficacy when compared to calcium hydroxide.

CONCLUSION:Nutmeg shows promising results for the use as an antibacterial intracanal medicament.

Comment [DAL1]: Don't separate!!

Keywords –Agar diffusion method, Zone of inhibition, Nutmeg, Calcium hydroxide

INTRODUCTION:

Caries is a global public health challenge and is characterized as a dynamic process. Caries develops when there is a susceptible tooth exposed to pathogenic flora in the presence of substrate. Under these conditions, the bacteria metabolize the substrate to form acid, which decalcifies teethstarting with enamel followed by dentin and if left untreated it may progress to the pulp.

Comment [DAL2]: Where did you found this information?In the literature? Please indicate an author!

Bacteria and their by-products are mainly responsible for dental pulp necrosis and periapical lesions requiring root canal treatment.*Enterococcus faecalis* is a very big challenge to the success of root canal therapy. It is the most common microorganism responsible for recurrent infections and resistant endemic infections.*E. faecalis* is found in 22–77% of failed cases [1]and have found to have high resistance to high pH and concentrations of saline solution and also has a high ability for biofilm formation which is said to be one of the most important virulence factors. [2]

The ultimate goals of endodontic treatment are complete removal of bacteria from infected root canals and the complete seal of disinfected root canals. Intracanal medicaments have been thought an essential step in killing the bacteria in root canals.[3]Calcium hydroxide has

been used as golden standard for intracanal medicament due to its antimicrobial activity, but is still far from an ideal intracanal medicament. [4]

Ayurveda originated in India 5000 years ago. There is renewed interest in herbal products in recent years as it demonstrated antibacterial, antiseptic, and analgesic properties. [5] In an attempt to search for new materials, we have tried to amalgamate modern technology and understanding with the old but time-tested views of Ayurveda.

The aim of this study is to compare, invitro, the antibacterial efficacy of combination of herbal extracts, i.e nutmeg + aloe vera, nutmeg + neem and nutmeg + distilled water with calcium hydroxide as an intracanal medicament on Enterococcus faecalis.To our best knowledge there are no studies on the combinations of the above-mentioned products and hence in this study we have combined nutmeg with aloe vera and neem to see if their combination shows synergistic or antagonistic effect against *E. faecalis.*

MATERIALS AND METHOD:

This study is an invitro pilot study. This study was carried out in the Infexn Laboratories located in Thane district of Maharashtra, India. Ethical clearance was taken from the Faculty Research Committee of D. Y. Patil University School of Dentistry (Approval number - IREB/2022/PEDO/15).

Materials used in our study include nutmeg powder, neem oil and aloe vera extract.

Mature fresh aloe vera leaves were washed with water and the thick upper epidermis layer was removed. The mucilaginous solid gel was scrapped from the leaves and was collected in the sterile glass container. [6] Nutmeg powder and neem oil was purchased from an Ayurvedic shop in its pure form without preservatives.

In the laboratory, Enterococcus faecalis ATCC 29212 bacterial strain was used (fig.1).*E. faecalis* was inoculated on one Mueller Hinton agar plate (fig.2). On this plate four wells of about 8-10mm in diameter were made with help of a sterile cork tip (fig.3) and combinations of the stated herbal extractswere added in these wells. [7]

On a clean sterile glass slab, 1.2g of nutmeg powder was dispensed then 0.2ml of Liquid (Aloe vera, neem oil and distilled water) was mixed*(Table.1.)*. Currently most used intracanal medicament is calcium hydroxide which is in a paste form. The combination of materials that we made are to be tested as intracanal medicaments hence, after several permutations and combinations, the ratio of powder (nutmeg) and liquid (neem oil, aloe vera and distilled water) was decided*(Table.1.)*to have a similar consistency as that of calcium hydroxide used as an intracanal medicament. This was done to have ease of carrying the material into the canals similar to that of calcium hydroxide.

A Mueller Hinton agar plate in which 4 wells were made, we added nutmeg + aloe vera, nutmeg + neem oil, nutmeg + distilled water and calcium hydroxide, respectively.The plate was then incubated at 37 degrees Celsius for 48hours.

STATISTICALA ANALYSIS:

The zone of inhibition of all 4 wells was manually measured using a metered scale and compared.

RESULTS:

After 48hours the zone of inhibition was observed and measured.it was seen thatmaximum zone of inhibition of 24mm was shown by Nutmeg + distilled water followed by Nutmeg + Aloe vera (20mm), Nutmeg + Neem (19mm) and minimum zone of inhibition was shown by Calcium hydroxide which was 15mm. *(Table.2.).* We observed that Nutmeg + distilled water

showed a better zone of inhibition followed by Nutmeg + Aloe vera, Nutmeg + Neem oil and Calcium hydroxide which was the least (fig.4).

DISCUSSION:

Elimination of bacteria by proper cleaning and shaping is the main objective of a root canal treatment.Intracanal medicament with good antimicrobial activity is desired to eliminate the residual microorganisms in the canals.

Enterococcus faecalis is a grampositive facultative anaerobe and was chosen to be tested as it is the most resistant species in the oral cavity and is the possible reason for occurrence of failure of root canal treatment.[8]*E. faecalis*has an inherent ability to tolerate starvation, high pH, high salt concentration, biofilm formation and resistance to antibiotics due to which its eradication is more challenging.[9]Therefore there is a strong need to investigate an intracanal medicament which can eliminate it.

Myristica fragrans Houtt (Myristicaceae family) also called nutmeg, is the seed kernel inside the fruit and mace is the fleshy red which appears as net like skin covering on the kernel. [10]Active compounds present in *Myristica fragrans* include myristicin, trimyristicin and myristic acid. These have –COOH, -COOR, NH2 and SH functional groups which have been suggested to be responsible for the antibacterial activity of *Myristica fragrans.*[11]*Shafiei et al*in his study stated that M.fragrans is effective against gram positive cariogenic and gram negative periodontopathic bacteria and he also has confirmed its broad spectrum antibacterial activity.[8]*Setty et al*also showed that *M.fragrans* has excellent antimicrobial activity against endodontic pathogens of primary teeth.[9]Nutmeghas been suggested to have analgesic, anti-

inflammatory and antibacterial activities which has been showed similarly in the present study where Nutmeg + distilled water has shown the largest zone of inhibition when compared to calcium hydroxide.

Aloe vera belongs to Liliacea family. It is a cactus-like plant that grows readily in hot and dry climates. The parenchymatous cells found in the leaves of fresh Aloe Vera tend to secrete a colorless mucilaginous gel which contains 98-99% water and 1-2% active compounds like Aloesin, Aloin, Aloe-emodin, Aloe-mannan, Flavonoids, Saponin, Sterols, Amino acids and Vitamins.[4]Negin Ghasemi et al. in his study showed that Aloe vera can inhibit the growth of *E. faecalis* equal with common antimicrobial agents and it can be used as a natural antimicrobial material in the intracanal medicament to reduce infection.[12]Azadirachta indica, known as neem has a vast array of biologically active compounds which are chemically diverse and structurally multifaceted.[13]Mustafa. M in his study stated that neem leaf extract has significant antimicrobial activity against *E. faecalis* and can be used as an intracanal medication. [14]Aloe vera and neem in this present study were used as a vehicle to mix with nutmeg powder to a similar consistency as that of calcium hydroxide and to observe whether these combinationsof herbal productsshow a synergistic antibacterial efficacy or not.

CONCLUSION:

1.In this study when aloe vera and neem were mixed with nutmeg powder, we observed that there was decrease in antimicrobial effect of nutmeg

2. Nutmeg alone had shown better antimicrobial efficacy against *E. Faecalis* as compared to its combination with aloe vera and neem which in turn were still better when compared to calcium hydroxide.

There is paucity of studies with respect to nutmeghence furtherstudies to evaluate its efficacy against other organisms in the oral cavity can be studied.Use of nutmeg as a mouthwash, as an irrigant or an obturating material can also be evaluated.

The promising results of our study with nutmeg can open new vistas in finding new products with antibacterial effect as intracanal medication.

LIMITATIONS:

We have checked the antibacterial effects of nutmeg and combination against only one organism. Since endodontics involves multiple bacteria and multiple strains, we need to explore the prospects further.

In this study, we have only done preliminary screening using the agar diffusion method of our herbal combinations to see if they have antibacterial efficacy against *E. faecalis*.

REFRENCES:

1. M. Janani, F. Jafari, M. Samiei et al., "Evaluation of antibacterial efficacy of photodynamic therapy vs. 2.5% NaOCl against E. faecalis-infected root canals using real-time PCR technique," Journal of Clinical and Experimental Dentistry, vol. 9, no. 4, pp. e539–e544, 2017.

2. H. S. Kafil, A. M. Mobarez, and M. F. Moghadam, "Adhesion and virulence factor properties of enterococci isolated from clinical samples in Iran," Indian Journal of Pathology and Microbiology, vol. 56, no. 3, p. 238, 2013.

3.Kawashima N, Wadachi R, Suda H, Yeng T, Parashos P. Root canal medicaments. Int Dent J. 2009 Feb;59(1):5-11. PMID: 19323305.

4.Ghatole K, Gowdra RH, Azher S, Sabharwal S, Singh VT, Sundararajan BV. Enhancing the antibacterial activity of the gold standard intracanal medicament with incorporation of silver zeolite: An in vitro study. J Int Soc Prev Community Dent. 2016 Jan-Feb;6(1):75-9.

5.Torwane NA, Hongal S, Goel P, Chandrashekar BR. Role of Ayurveda in management of oral health. Pharmacogn Rev. 2014 Jan;8(15):16-21.

6.Jain S, Rathod N, Nagi R, Sur J, Laheji A, Gupta N, Agrawal P, Prasad S. Antibacterial Effect of Aloe Vera Gel against Oral Pathogens: An In-vitro Study. J Clin Diagn Res. 2016

Nov;10(11):ZC41-ZC44. doi: 10.7860/JCDR/2016/21450.8890. Epub 2016 Nov 1. PMID: 28050502; PMCID: PMC5198455.

7.Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. J Pharm Anal. 2016 Apr;6(2):71-79. doi: 10.1016/j.jpha.2015.11.005. Epub 2015 Dec 2. PMID: 29403965; PMCID: PMC5762448.

8.Devi MT, Saha S, Tripathi AM, Dhinsa K, Kalra SK, Ghoshal U. Evaluation of the Antimicrobial Efficacy of Herbal Extracts Added to Root Canal Sealers of Different Bases: An *In Vitro* Study. Int J Clin Pediatr Dent. 2019 Sep-Oct;12(5):398-404. doi: 10.5005/jp-journals-10005-1660. PMID: 32440044; PMCID: PMC7229369.

9.Saha S, Nair R, Asrani H. Comparative Evaluation of Propolis, Metronidazole with Chlorhexidine, Calcium Hydroxide and Curcuma Longa Extract as Intracanal Medicament Against E.faecalis- An Invitro Study. J Clin Diagn Res. 2015 Nov;9(11):ZC19-21. doi: 10.7860/JCDR/2015/14093.6734. Epub 2015 Nov 1. PMID: 26673857; PMCID: PMC4668516.

10.Shafiei Z, Shuhairi NN, Md Fazly Shah Yap N, Harry Sibungkil CA, Latip J. Antibacterial Activity of Myristica fragrans against Oral Pathogens. Evid Based Complement Alternat Med. 2012;2012:825362. doi: 10.1155/2012/825362. Epub 2012 Aug 28. PMID: 23049613; PMCID: PMC3434417.

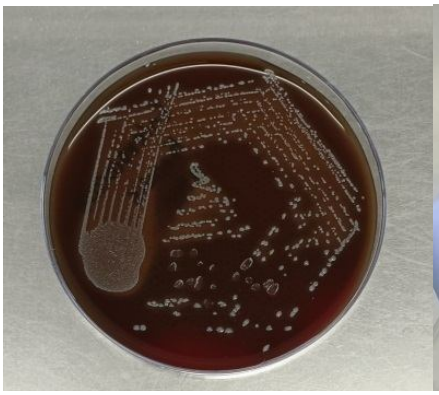
11.Setty JV, Srinivasan I, Sathiesh RT, Kale M, Shetty VV, Venkatesh S. *In vitro* evaluation of antimicrobial effect of *Myristica* fragrans on common endodontic pathogens. J Indian Soc Pedod Prev Dent. 2020 Apr-Jun;38(2):145-151. doi: 10.4103/JISPPD.JISPPD_214_20. PMID: 32611860.

12.Ghasemi N, Behnezhad M, Asgharzadeh M, Zeinalzadeh E, Kafil HS. Antibacterial Properties of *Aloe vera* on Intracanal Medicaments against *Enterococcus faecalis* Biofilm at Different Stages of Development. Int J Dent. 2020 Dec 28;2020:8855277. doi: 10.1155/2020/8855277. Erratum in: Int J Dent. 2021 Jun 10;2021:9825216. PMID: 33488716; PMCID: PMC7803133.

13.Lekshmi NC, Sowmia N, Viveka S, Brindha JR, Jeeva S. The inhibiting effect of Azadirachta indica against dental pathogens. Asian J Plant Sci Res. 2012;2(1):6-10.

14.Mustafa M. Antibacterial Efficacy of Neem (Azadirachta indica) Extract against Enterococcus faecalis: An in vitro Study. J Contemp Dent Pract. 2016 Oct 1;17(10):791-794. doi: 10.5005/jp-journals-10024-1932. PMID: 27794147.

FIGURES:



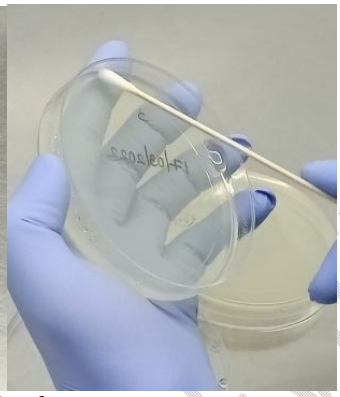


Fig. 1 Mueller Hinton agar plateFig.2 Sterilization

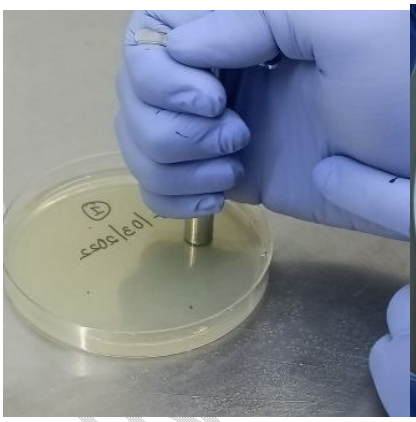


Fig. 3 Detection technique Fig. 4 – zone of inhibition

TABLES:

Table.1. Herbal combinations

Sr.no	HERBAL COMBINATIONS (powder+liquid)
1.	1.2g Nutmeg powder + 0.2ml Aloe vera extract
2.	1.2g Nutmeg powder + 0.2ml Neem seed oil
3.	1.2g Nutmeg powder + 0.2ml Distilled water

Table.2.- Zone of inhibition of different combinations (in mm)

Sr.no	Combination of herbal extracts	Zone of inhibition (mm)
1.	Nutmeg + Aloe vera	20
2.	Nutmeg + Neem Oil	19
3.	Nutmeg + Distilled water	24
4.	Calcium hydroxide	15