

***In vitro* comparative study of the antimicrobial efficacy of combined nutmeg, aloe vera and neem as an intracanal medicament with calcium hydroxide on enterococcus faecalis**

ABSTRACT:

BACKGROUND: Ideal requirement of an intracanal medicament is that it should have an antibacterial action for the success of endodontic procedures. The "golden standard" intracanal medication is calcium hydroxide, but it still has drawbacks like internal root resorption. The search for an ideal intracanal medication that works well is an ongoing process that is still unsuccessful. In this pilot study, we attempted to search for new materials by drawing motivation from the earlier time. Ayurveda, started in India 3000 years back. Since many herbal products have demonstrated antibacterial, antiseptic, and analgesic properties, there has been renewed interest in them in recent years.

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 ATCC strain 29212 of the bacterium *E. faecalis* was used in the study. Mueller Hinton agar plate was vaccinated with *E. faecalis*. The herbal extract mixtures were pipetted into the agar wells on this agar plate, which had four wells with diameters ranging from 8 to 10 millimeters. After using calcium hydroxide as a control, which is the gold standard for intracanal medication, the plate was incubated. The width of the restraint zones around every one of the wells were estimated physically after hatching for 48hrs.

RESULTS: The 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 its use as an antibacterial intracanal medicament.

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

INTRODUCTION:

“Caries is a global public health problem with a dynamic process. When a susceptible tooth is exposed to pathogenic flora in the presence of substrate, caries develops. Under these conditions, the bacteria metabolize the substrate to produce acid, which decalcifies the enamel, dentin, and pulp of the teeth if left untreated”.^[1]

The majority of dental pulp periapical infections that necessitate root canal treatment are caused by bacteria and their byproducts.^[2] Root canal therapy is extremely susceptible to *Enterococcus faecalis*. It is the most prevalent microorganism that causes resistant endemic infections and recurrent infections. The bacterium *E. faecalis* is present in 22–77% of unsuccessful cases^[3] and has been proven to be highly resistant to high pH and saline solution concentrations. It also has a high capacity for biofilm formation, which is considered to be one of the most significant virulence factors.^[4]

“The complete sealing of disinfected root canals and the complete removal of bacteria from infected root canals are the ultimate objectives of endodontic treatment. Intracanal medicaments have been believed to be a fundamental stage in killing the microscopic organisms in root canals”.^[5] “Calcium hydroxide is one of the commonly used material as intracanal medicament due to its antimicrobial activity, but yet, is still far from an ideal intracanal medicament”.^[6]

“Ayurveda dates back 5000 years to India. There is reestablished interest in herbal products lately as it exhibited antibacterial, disinfectant, and pain relieving properties”.^[7] In an attempt to search for new materials, an amalgamation of modern technology and understanding with the old but time-tested views of Ayurveda has been proposed.

This study aims to compare the antibacterial efficacy of nutmeg + aloe vera, nutmeg + neem, and nutmeg + distilled water with calcium hydroxide as an intracanal medication against *Enterococcus faecalis* in vitro. Nutmeg, aloe vera, and neem have been combined in this study to see if they have a synergistic or antagonistic effect against *E. faecalis*. To the best of our knowledge, there are no studies on the combination of the above mentioned products.

MATERIALS AND METHOD:

This is a pilot in vitro study. This study was conducted in the Infexn Laboratories located in the Thane district of Maharashtra, India. The Faculty Research Committee of the D. Y. Patil University School of Dentistry granted ethical approval (approval number IREB/2022/PEDO/15).^[8]

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

“The thick upper epidermis layer was removed from mature fresh aloe vera leaves by washing them with water. The sterile glass container contained the mucilaginous solid gel that was removed from the leaves”.^[9] 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* strain was inoculated on one Mueller Hinton agar plate^[8] (fig.2). On this plate, 4 wells of about 8-10mm in diameter were made with help of a sterile cork tip (fig.3) and combinations of the stated herbal extracts were added in these wells.^[10,8]

On a clean sterile glass slab, 1.2grams of nutmeg powder and 0.2ml of Liquid (Aloe vera, neem oil and distilled water) was dispensed and mixed (Table.1.). As of now most utilized intracanal medicament is calcium hydroxide which is in a paste form. The materials we created will be tested as intracanal medications, so the ratio of powder (nutmeg) to liquid (neem oil, aloe vera, and distilled water) was chosen after several permutations (Table.1). to have a consistency that was comparable to that of calcium hydroxide that was used as an intracanal medication. This was done so that the material could be easily carried into canals like calcium hydroxide. [8]

To a Mueller Hinton agar plate in which 4 wells were made, certain quantity of nutmeg + aloe vera, nutmeg + neem oil, nutmeg + distilled water and calcium hydroxide were respectively added. [8] The plate was then incubated at 37 degrees Celsius for 48 hours.

STATISTICAL ANALYSIS:

The zone of inhibition of all 4 wells was manually measured using a metered scale. Only one agar plate was used in which 4 wells were made. Hence, the well with a particular combination showing the maximum zone of inhibition was considered to have maximum antibacterial efficacy than the other combinations.

RESULTS:

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

The primary objective of a root canal treatment is to get rid of bacteria through proper cleaning and shaping. In order to eliminate any remaining microorganisms in the canals, an intracanal medication with strong antimicrobial activity is desired.

“*Enterococcus faecalis*, a gram positive facultative anaerobe was chosen to be tested as it is the most resistant species in the oral cavity and is the possible reason for the occurrence of failure of root canal treatment”.^[11] “*E. faecalis* has an innate capacity to endure starvation, high pH, high salt concentration, biofilm formation and protection from anti-infection agents because of which its eradication is more challenging”.^[12] 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 found inside the fruit, while mace is the fleshy red material that resembles a net-like skin covering.¹³ Myristicin, trimyristicin, and myristic acid are among the active substances found in *Myristica fragrans*. These have the functional groups -COOH, -COOR, NH₂, and SH, which have been theorised to be in charge of *Myristica fragrans*' antibacterial action.^[14] According to Shafiei et al., who also validated *M. fragrans*' broad spectrum antibacterial activity, the organism is efficient against both gramme positive and gramme negative pathogenic bacteria.^[13] *M. fragrans* has strong antibacterial action against endodontic infections of primary teeth, as demonstrated by Setty et al.^[14] Nutmeg has 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 the Liliacea family. It is a plant that resembles a cactus that thrives in hot, dry areas. Fresh Aloe Vera leaves have parenchymatous cells that release a colourless,

mucilaginous gel that is 98–99% water and includes one to two percent of active ingredients such as aloesin, aloin, aloe-emodin, aloe-mannan, flavonoids, saponin, sterols, amino acids, and vitamins.^[6] Aloe vera can suppress the growth of *E. faecalis* together with standard antimicrobial medicines, and it can be utilised as a natural antibacterial substance in the intracanal medication to minimise infection, according to a research by Negin Ghasemi et al.^[15] Neem also known as *Azadirachta indica*, has a vast array of biologically active compounds which are chemically diverse and structurally multifaceted.^[16] Neem leaf extract has considerable antibacterial action against *E. faecalis*, according to study done by Mustafa M.^[17] 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 combinations of herbal products show a synergistic antibacterial efficacy or not.

CONCLUSION:

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

2. Nutmeg alone had shown a 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 a paucity of studies with respect to nutmeg hence further studies to evaluate its efficacy against other organisms in the oral cavity can be carried out. The 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 an intracanal medication.

LIMITATIONS:

The antibacterial effect of nutmeg and its combinations against only one organism was checked. Since endodontics involves multiple bacteria and multiple strains, the prospects need to be explored further.

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

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CONFLICT OF INTERESTS:

No conflicts of interests were seen.

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FIGURES:

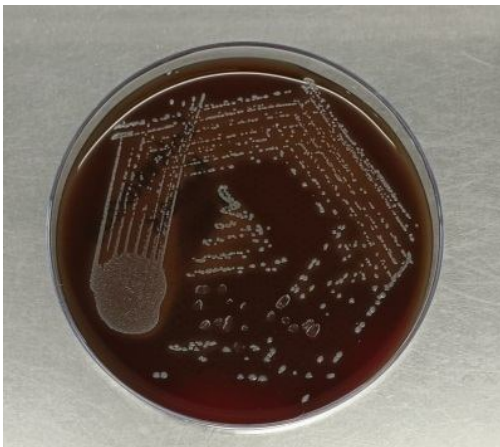


Fig. 1 Mueller Hinton agar plate

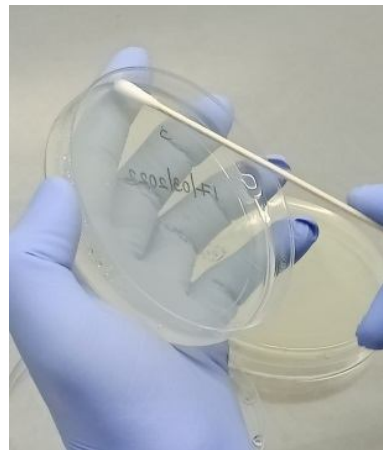


Fig.2 Sterilization



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