

# COMPARISON OF THE ANTIMICROBIAL EFFICACY OF COMBINATIONS OF TURMERIC, ALOE VERA AND NEEM AS AN INTRACANAL MEDICAMENT WITH CALCIUM HYDROXIDE ON ENTEROCOCCUS FAECALIS – AN INVITRO STUDY

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## ABSTRACT

Intracanal medicament is an integral part of endodontic procedures that needs to have antibacterial effect on the infected root canal bacteria for the success of endodontic procedures. Calcium hydroxide is currently the 'golden standard' intracanal medicament. The search for an effective and ideal intracanal medicament is ongoing process that is still elusive. In an attempt to search for new materials in future, we, in this study are trying to look at 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 invitro the antibacterial efficacy of a combination of herbal extracts, i.e. turmeric + Aloe vera, turmeric + neem, turmeric + distilled water with calcium hydroxide as an intracanal medicament on *Enterococcus faecalis*.

**MATERIALS AND METHOD:** This study is an invitro pilot study in which *E. Faecalis* bacterial ATCC strain – 29212 was used. *E. Faecalis* is a facultative anaerobe most commonly found in an infected root canal. A Mueller Hinton agar plate was inoculated with *E. faecalis* . Wells with diameter 6-8mm were punched and the Herbal extract combinations were pipetted into these agar wells. The diameter of the inhibition zones around all the wells were measured manually after incubation for 48hrs. The zones of inhibition of turmeric combinations were then compared to the zone of inhibition of calcium hydroxide.

**RESULTS:** No Zone of inhibition (0mm) was seen around the wells containing Turmeric and its combinations. Calcium hydroxide (17mm) showed better antibacterial efficacy when compared to turmeric and its combinations.

**CONCLUSION:** Turmeric showed no antibacterial efficacy against *E.faecalis* indicating *E.faecalis* ATCC strain 29212 was resistant to turmeric and its combinations with aloe vera and neem.

**Keywords** – Ayurveda, Agar diffusion method, Zone of inhibition

## **INTRODUCTION**

Caries is a global public health challenge and is characterized as a dynamic process of demineralization of inorganic and organic structures of teeth starting with enamel followed by dentin and if left untreated it may progress to the pulp. Bacteria and their by-products are mainly responsible for dental pulp necrosis and periapical lesions requiring root canal treatment. Many bacteria for example *S.mutans*, *S.salivarius*, *Lactobacilli*, *Actinomyces* and *E.faecalis* are commonly seen in the oral cavity. Of these, *E.faecalis* is the most resistant species in the oral cavity and the possible reason for failure of root canal treatment.<sup>[1]</sup>

The ultimate goal of endodontic treatment is a complete removal of bacteria, their by-products and pulpal remnants from infected root canals and the complete seal of disinfected root canals. Intracanal medicaments have been thought an important step in eliminating the bacteria present in the root canals.<sup>[2]</sup> Calcium hydroxide has been used as a gold standard material for intracanal medicament due to its antimicrobial activity, but is still far from being an ideal intracanal medicament.<sup>[3]</sup>

Ayurveda is the ancient Indian system of health-care and longevity. It involves a holistic view of man, his health and illness. There are approximately 1250 Indian medicinal plants that are used in formulating beneficial concoctions according to Ayurveda and many more that are still to be discovered. This 5000-year-old system of medicine recommends treatments with

specific herbs and minerals to cure various diseases. <sup>[4]</sup> In recent years, there has been a renewed interest in herbal products as it has demonstrated antibacterial, antiseptic, and analgesic properties with limited if any side-effects. In an attempt to search for new materials, we have tried to amalgamate modern technology and understanding with the old, but time-tested benefits of Ayurveda. <sup>[5]</sup>

The aim of this study is to compare invitro the antibacterial efficacy of combination of herbal extracts, i.e., turmeric + distilled water, turmeric + Aloe vera, turmeric + neem 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 turmeric 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 turmeric powder, neem oil, aloe vera extract and distilled water. 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. <sup>[6]</sup> Turmeric powder and neem oil was purchased from an Ayurvedic shop in pure form without preservatives.

*Enterococcus faecalis* ATCC- 29212 bacterial strain was used (fig.1). *E.faecalis* was inoculated on one Mueller Hinton agar plate and four wells of about 8-10mm in diameter were made with help of a sterile cork tip. (fig.2, fig.3) <sup>[7]</sup> Combinations of our herbal extracts were added in these wells. <sup>[8]</sup>

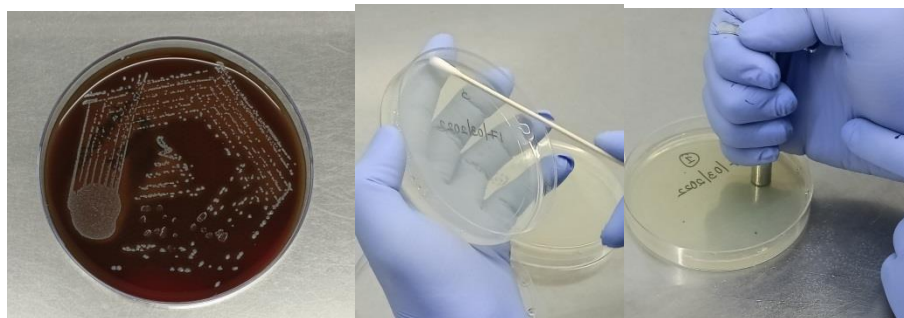


Fig.1.

Fig.2.

Fig.3.

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

*Table.1. Herbal Combinations*

On a clean sterile glass slab, 1.2g of turmeric powder was dispensed then 0.2ml of Liquid (Aloe vera, neem oil and distilled water) was mixed. 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 (turmeric) and liquid (neem oil, aloe vera and distilled water) was decided. It was made to have a similar consistency as that of calcium hydroxide used as an intracanal medicament. This was done so as to have ease of carrying the material into the canals similar to that of calcium hydroxide.

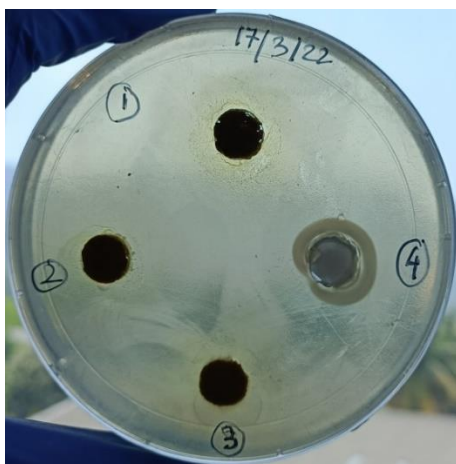
On one Mueller Hinton agar plate in which 4 wells were made, we added turmeric + aloe vera, turmeric + neem oil, turmeric + distilled water and calcium hydroxide, respectively. The plate was then incubated at 37 degrees Celsius for 48hrs.

### **STATISTICAL ANALYSIS**

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

### **RESULTS**

After 48hrs, the plate was removed from the incubator.



We observed no zone of inhibition around the wells containing Turmeric + Aloe vera, Turmeric + Neem oil and Turmeric + distilled water (fig.4). Growth of *E. faecalis* colonies were observed around the wells indicating resistance of *E. faecalis* to turmeric and its combinations (fig.5). Calcium hydroxide showed better antibacterial efficacy as compared to turmeric.



Fig. 5.

Sr.no	Combination of herbal extracts	Zone of inhibition (mm)
1.	Turmeric + Aloe vera	0
2.	Turmeric + Neem Oil	0
3.	Turmeric + Distilled water	0
4.	Calcium hydroxide	17

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

**DISCUSSION**

The main goal of root canal treatment is to achieve maximum disinfection of the root canals, which is a challenging task owing to the complex anatomy of the root canals. The number of

bacteria are reduced partly by shaping the canals and predominantly by the use of intra-canal medicaments.<sup>[9]</sup> An intracanal medicament with good antimicrobial activity is needed to eliminate the residual microorganisms in the canals.

*Enterococcus faecalis*, a gram-positive facultative anaerobe was chosen as the test organism as it is found to be the most resistant species in the oral cavity and the possible reason for failure of root canal treatment.<sup>[1]</sup> *Enterococcus faecalis* is the most commonly isolated species from the canals of teeth presenting post-treatment diseases. *E. faecalis* has been reported in 10-30% of root canal failures, inflamed root canals and in untreated canals.<sup>[10]</sup> Considering the resistance of *E. faecalis*, there is a strong need to investigate intracanal medicaments which can eliminate it.

Turmeric (*haldi*), a rhizome of *Curcuma longa*, is a flavourful yellow-orange spice. Its plant is 3 feet in height and has lance-shaped leaves and spikes of yellow flowers that grow in a fleshy rhizome or in underground stem. An orange pulp contained inside the rhizome constitutes the source of turmeric medicinal powder.<sup>[11]</sup> Components of turmeric are named curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin. Curcumin (diferuloylmethane) is a polyphenol derived from *Curcuma longa* plant, commonly known as turmeric. The active constituents of turmeric are the flavonoid curcumin (diferuloylmethane) and various volatile oils including tumerone, atlantone, and zingiberone.<sup>[12]</sup> A study carried by Shruti Saha et al evaluated antimicrobial efficacy of Calcium Hydroxide and Curcuma Longa Extract as intracanal medicament against *E. faecalis* and concluded that Curcuma longa has better antimicrobial efficacy than Calcium hydroxide as an intracanal medicament against *E. faecalis*.<sup>[13]</sup> Rakesh Kumar Yadav et al compared antimicrobial efficacy of calcium hydroxide, chlorhexidine gel, and curcumin against *E. faecalis* and concluded that C. longa extract showed mild activity against *E. Faecalis* while calcium hydroxide paste was the least efficient in eliminating *E. faecalis*.<sup>[14]</sup> But contradictory to the above mentioned study, Yang et al in his study concluded that turmeric exhibited poor antibacterial properties, with a higher rate of bacterial growth<sup>[15]</sup> which was similar to the present study where growth of *E.faecalis* was seen surrounding the wells with turmeric + distilled water, turmeric + neem and turmeric + aloe vera suggesting that *E.faecalis* is resistant to turmeric and its combinations.

Aloe vera belongs to Liliacea family which is a cactus-like plant that grows readily in hot, dry climates. Aloe vera is a cactus-like plant that contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids. It has

anti-inflammatory, anti-arthritic activity, antibacterial, and hypoglycemic effects.<sup>[16]</sup> Goud S et al. in his study concluded that aloe vera can be used as an antibacterial agent in novel drugs for the treatment of bacterial diseases.<sup>[17]</sup> *Azadirachta indica* has several active constituents like nimbidin, nimbin, nimbolide, gedunin, azadirachtin, mahmoodin, margolone, and cyclictrisulfide which are responsible for its antibacterial action.<sup>[18]</sup> All parts of the neem tree-leaves, flowers, seeds, fruits, roots and bark have been used conventionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders.<sup>[19]</sup> Mohammed Mustafa in his study concluded that neem leaf extract has significant antimicrobial activity against *E. faecalis* and opens the perspectives for the use of neem extract as an intracanal medication.<sup>[20]</sup> Aloe vera and neem oil obtained from seeds in this present study was used as a vehicle to mix with turmeric powder to a similar consistency as that of calcium hydroxide and to observe whether these combinations show a synergistic or antagonist antibacterial efficacy. In this study when aloe vera and neem were mixed with turmeric powder, they did not show any antibacterial activity against *E.faecalis*.

### LIMITATIONS

We have checked the antibacterial effects of turmeric 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. The combinations we made did not show any antibacterial efficacy. As none of the combinations showed zone of inhibition, we have not done the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and cytotoxicity tests, which was to be the second phase of the study.

It might be due to the percentage of curcumin present. Hence, we plan to study turmeric from different regions that have different percentages of curcumin, so our next step would be to compare antibacterial efficacy of turmeric from different regions, the turmeric which would show better antibacterial efficacy would be further taken up for MIC, MBC and cytotoxicity tests.

### CONCLUSION

Turmeric showed no antibacterial efficacy against *E.faecalis* indicating *E.faecalis* ATCC strain 29212 resistant to turmeric and its combination with aloe vera and neem.

There are various types of curcumin found in different states of India with varied curcumin percentages. Also, the soil and other minerals may affect the active content in turmeric. Hence to come to a more conclusive result about efficacy of turmeric; we propose studies

with curcumin from different locations e.g Wai, Salem and Meghalaya from India and compare their antibacterial properties.

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