



## "ANTIMICROBIAL EFFICACY OF TRIPHALA AND CURCUMIN EXTRACT IN COMPARISON WITH CALCIUM HYDROXIDE AGAINST E. FAECALIS AS AN INTRACANAL MEDICAMENT AN IN VITRO STUDY"

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### ABSTRACT

The purpose of this study was to evaluate, in vitro, the antimicrobial efficacy of Triphala and Curcumin extract in comparison with calcium hydroxide against *E. faecalis*. The agar well diffusion test was used to check the antimicrobial activity of the extracts of the medicinal plants along with  $\text{Ca}(\text{OH})_2$ . Three different concentrations of the tested agents were used for the study. The values of Zone of Inhibition were tabulated according to the concentration of the tested agent and data was statistically analyzed using one way ANOVA test of significance  $p < 0.05$ . The Minimum Inhibitory Concentration (MIC) values were also recorded. There was statistically significant difference in the values of zone of inhibition of calcium hydroxide, Triphala and Curcumin. All the plants extracts showed considerable antimicrobial activity against *E. faecalis*. At 1 gm. concentration,  $\text{Ca}(\text{OH})_2$  was most effective against *E. faecalis*. Hence there is possibility of use of  $\text{Ca}(\text{OH})_2$  which can be substituted with Curcumin or Triphala as an intracanal medicament.

### KEYWORDS

#### INTRODUCTION:

The major objective in root canal treatment is to disinfect the entire root canal system. Although cleaning and shaping and use of antimicrobial medicaments are effective in reducing the bacterial load but some bacteria do remain behind and multiply, causing reinfection of the canal. Considering the ineffectiveness, potential side effects and safety concerns of synthetic drugs, the herbal alternatives for endodontic usage might prove to be advantageous.

Several studies of endodontically treated teeth requiring re-treatment have shown a prevalence of *Enterococcus faecalis* which is a fermentative, facultative anaerobic, Gram positive coccus. It possesses many virulence factors which makes it capable of re-infecting the obturated canal.<sup>1</sup>

The increase in antibiotic resistant strains caused by synthetic drugs led to search for herbal irrigants like Triphala, Curcumin etc. They are good chelating agents and hence may aid in removal of smear layer. The major advantages of using herbal alternatives are easy availability, low toxicity and lack of microbial resistance.<sup>1</sup>

The present study is intended to evaluate the antimicrobial Efficacy of different herbal derivatives like Triphala and Curcumin in comparison with calcium hydroxide against *E. faecalis* as an intracanal medicament.

#### MATERIALS & METHODS:

##### Medicaments tested

1. Calcium hydroxide powder with saline.
2. Curcumin (100% pure) with dimethyl sulfoxide (DMSO)
3. Triphala extract. (Zandu pharmaceuticals)

##### Microbiological media used

1. BHI (brain heart infusion) broth (HI-MEDIA PVT.LTD, MUMBAI)
2. Nutrient agar
3. Normal saline.

The armamentaria were sterilized by autoclaving at 121°C, 15 psi for 20 minutes.

##### Test organism and inoculation preparation

- Standard strain of *E. faecalis* was obtained. Single colony of *E. faecalis* from BHI agar plate was inoculated into BHI broth. The broth was then incubated at 37°C for 24 hours to obtain bacterial suspension.
- Determination of Minimum Inhibitory Concentration (MIC)
- MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration).
- In this method, the broth dilution technique was utilized where the rhizome extract was prepared to the highest concentration of 1g/ml (stock concentration) in DMSO and serially diluted to a working concentration ranging from 500mg/ml to 1g/ml using dimethyl sulfoxide for the herbal samples and normal saline for calcium hydroxide.
- These test tubes were then inoculated with 0.1 ml suspension of the test organisms.
- After 24 hours of incubation at 37 °C, the test tubes were observed for turbidity. The least concentration where no turbidity is observed was determined and noted as the minimum inhibitory concentration (MIC) value.

##### Antimicrobial activity assay

- Agar well diffusion method was used to conduct the antimicrobial susceptibility test; three different concentrations of the prepared samples were made.
- The samples were then divided into five experimental groups.

**Group I:** Curcumin- The three concentrations of curcumin were 1g/ml, 750mg/ml, and 500mg/ml.

**Group II:** Triphala- (Zandu pharmaceuticals) The aqueous product of Triphala churna was prepared in three concentrations: 1g/ml, 750mg/ml, and 500mg/ml.

**Group III:** Calcium hydroxide (HI-MEDIA PVT. LTD) powder was mixed with sterile saline in the ratio of 1.5:1 (wt. /vol) to obtain three concentrations (10µg/ml, 20µg/ml, 30µg/ml) in paste form.

**Group IV:** Control group in which DMSO is placed.

- Wells of 8 mm size were made with sterile borer into 12 agar plates containing the bacterial inoculum. The plates were then incubated at 37°C for 24 hours.
- 100µl volume of each experimental medicament prepared in three concentrations was dispensed into the wells of inoculated plates.
- DMSO was the solvent for preparation of different concentrations of the curcumin extract. It was introduced into one well as a control for solvent.
- After incubation for 24 hrs at 37 °C, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the extract.
- The bacterial strains grown were obtained in triplicates in each concentration of experimental medicaments.
- The zone of inhibition around each well of all the agar plates was measured and expressed in millimetres.

For statistical analysis, we have considered only two concentrations of each tested medicine.

Curcumin	0.5g/ml	1g/ml
Triphala	0.5g/ml	1g/ml
Calcium hydroxide	10µg/ml	20µg/ml

- The data obtained thus was analysed statistically by using one way ANOVA test.

**Results:**

The present in vitro study was conducted to compare the antibacterial efficacy of Curcumin and Triphala extracts with Calcium Hydroxide against E. Faecalis.

For the evaluation, the study protocol was designed as follows:

**Group 1** - Curcumin in three concentrations (1g/ml, 750mg/ml, 500mg/ml),

**Group 2** - Triphala in three concentrations (1g/ml, 750mg/ml, 500mg/ml),

**Group 3** -Calcium hydroxide in three concentrations (10µg/ml, 20µg/ml, 30µg/ml)

**Group 4** - DMSO (Dimethyl Sulfoxide)

The values of zone of inhibition were analyzed by one way ANOVA test. (Values for two concentrations of each medicament were considered for statistical analysis)

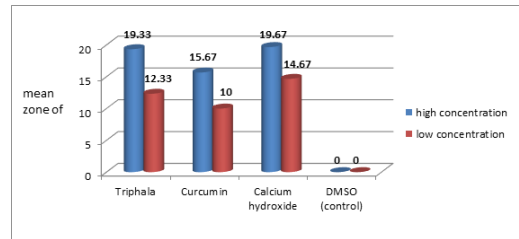
**Table no.1: Descriptive data of antibacterial activity of various agents against Enterococcus faecalis. (Agar well diffusion method)**

Agents	Concentration	Number (n)	Mean (zone of inhibition)	Standard deviation
Curcumin	1 g/ml	3	15.67	0.58
	0.5 g/ml	3	10.00	1.00
Triphala	1 g/ml	3	19.33	1.53
	0.5 g/ml	3	12.33	0.58
Calcium hydroxide	10 µl/ml	3	14.67	1.16
	20 µl/ml	3	19.67	0.58
DMSO (control)	-	3	0	0

Table 1 show that, the antibacterial property of calcium hydroxide is higher at both the concentrations followed by triphala.

Curcumin showed the lowest mean of zone of inhibition at both the concentrations indicating minimum antibacterial property.

**Figure no.1: Between group comparison of antibacterial activity of various agents against Enterococcus faecalis. (Agar well diffusion method)**



**Discussion**

In essence, endodontic infection is the infection of dental root canal system and is the major etiologic agent of apical periodontitis. Scientific evidence indicates that micro-organisms are essential to progression and perpetuation of pulpal and periapical diseases.<sup>2</sup>The success of endodontic treatment is directly related to the decrease in the number of root canal microorganisms. During root canal treatment, it is very important to control microbial agents by biomechanical procedures. In accordance with this concept, the main aim of root canal treatment is to eliminate bacteria and their irritants from root canals before filling.<sup>3</sup>

Mechanical debridement alone does not result in total or permanent reduction of bacteria.<sup>4</sup> Factors like anatomical complexities of root canal structures, organic residues and bacteria located in the dentinal tubules cannot be sufficiently cleaned, even after meticulous mechanical procedures.<sup>3</sup>Antimicrobial agents have been recommended as an adjunct to mechanical instrumentation to reduce the numbers of micro-organisms.<sup>4</sup>

If micro-organisms are allowed to remain in the root canal system at the time of filling, there is an increased risk of adverse outcome of endodontic treatment, which might change a simple primary infection to a more resistant secondary infection.<sup>4</sup>

Unlike primary infections a more restricted group of microbial species, E. faecalis has been more frequently detected in persistent or secondary infections associated with the unsatisfactory outcome of the endodontic treatment.<sup>5</sup>

In untreated root canals enterococci constitute only around 5% or less of total microflora.<sup>6</sup>Such results raises the question of how and when enterococci invade the root canal system. It can be hypothesized that E. faecalis could be present in untreated canals, but in such low numbers that it is not recovered. Due to the changes in root canal environment, this microorganism may grow to higher and recoverable proportions.<sup>6</sup>

Numerous studies showed that E. faecalis has some special characteristics that allow it to survive in conditions that are commonly lethal for many other microorganisms. These properties include an ability to grow in high salt concentrations, a wide temperature range, tolerance to a broad pH range, as well as resist their intracanal procedures.<sup>7</sup>

E. faecalis has the ability to resist high pH values and it is related to a functioning proton pump which drives protons into the cell to acidify the cytoplasm hence it has capacity to withstand a wide pH range up to around 11.5 of intracanal medicaments such as calcium hydroxide which is generally a highly potent antimicrobial dressing.<sup>8</sup>

Re-treatment requires the use of suitable intra-canal medicaments that will eliminate these bacteria, prevent their interappointment proliferation, act as a barrier against their ingress and cut off their nutrient supply.

Though calcium hydroxide has been the most effective intracanal medicament against a variety of microorganisms of the root canal flora, it has clearly been demonstrated to be ineffective against *E. faecalis*.<sup>9</sup>

The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs has also led to the search for herbal alternatives.

Turmeric (*Curcumin longa*) has been used for thousands of years as a medicinal herb. Curcumin, a phenolic compound has shown bactericidal properties in clinical testing with greater medicinal effects like antioxidant, anti-inflammatory, antimicrobial, antispasmodic, anticancer and many other properties which may prove to be a boon to dentistry.<sup>10</sup>

Triphala is a traditional ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants *Terminalia chebula*, *Terminalia bellerica* and *Phyllanthus embelica*, also known as 'three myrobalan'. Triphala means 'three' [tri] 'fruits' [phala].<sup>9</sup> It has a potential of anti-bacterial and anti-inflammatory activity.<sup>11</sup>

Keeping these above concepts in mind, this in vitro study was designed to compare the antibacterial efficacy of turmeric and Triphala extracts with calcium hydroxide against *E. faecalis*.

*E. faecalis* was selected in the present investigation, as it has been the most prevalent bacterial strain in the failed root canal system. Single colony of *E. faecalis* from BHI agar plate was inoculated into BHI broth. The broth was then incubated at 37°C for 24 hours to obtain bacterial suspension.

Minimum Inhibitory Concentration (MIC) of medicines to be tested was determined by broth dilution technique. The tested medicines were used in three different concentrations and were evaluated for antimicrobial effect for 24 hrs.

Curcumin	0.5g/ml*	0.75g/ml	1g/ml*
Triphala	0.5g/ml*	0.75g/ml	1g/ml*
Ca(OH) <sub>2</sub>	10µl/ml*	20µl/ml*	30µl/ml

\*: Concentrations considered for statistical analysis.

The results in the present study showed that calcium hydroxide has better antimicrobial properties than curcumin and Triphala at all three tested concentrations.

The mechanism of antibacterial action of curcuma and derivatives is not clear. Hypotheses have been proposed which involve hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, followed by partition in the lipid bilayer; perturbation of membrane permeability consequent to its expansion and increased fluidity causing the inhibition of membrane embedded enzymes; membrane disruption; destruction of electron transport systems and cell wall perturbation.<sup>12</sup> It is also suggested that curcumin, a polyphenolic compound strongly inhibits bacterial cell proliferation by inhibiting the assembly dynamics of FtsZ in the Z-ring needed for bacterial cell division. Curcumin has been shown to have a potent antibacterial activity against a number of pathogenic bacteria including *Enterococcus*.<sup>10</sup>

There can be several reasons for minimal antibacterial activity of curcumin in the present study against *E. faecalis*. The plant part used or type of extraction might have resulted in less activity or the time of collection of rhizomes and climate might have affected the amount of active constituents in the plant material.<sup>13</sup>

In the present study the second herbal medicine tested was Triphala and it showed better anti-bacterial property than curcumin at all concentrations. The available literature reveals that Triphala had been tested as an intracanal irrigant and not as an intracanal medicament.

The antibacterial effect of Triphala may be attributed to its formulation, which contains three different medicinal plants in

equal proportions, *Terminalia bellerica*, *Terminalia chebula*, and *Embelica officinalis*. In such formulations, different compounds may be of help in enhancing the potency of the active compounds resulting in an additive or synergistic positive effect. The strong antioxidant activity of Triphala may be partially responsible for many of the biological properties. *T. bellerica* was the most active antioxidant followed by *E. officinalis* and *T. chebula*. The major ingredients of *T. bellerica* are ellagic and gallic acid; *E. officinalis* has several gallic acid derivatives including epigallocatechingallate and in *T. chebulagallic acid* is the major ingredient. The presence of these active ingredients of phenolic nature may be responsible in scavenging free radicals generated by the bacteria. Tannic acid represents the major constituent of the ripe fruit of *T. chebula*, *T. bellerica* and *E. officinalis*.

In the present study calcium hydroxide showed best antibacterial activity at all 3 concentrations. The zone of inhibition observed on the agar plates was larger than that of Triphala and curcumin.

Calcium hydroxide inhibits microbial growth in canal due to its antimicrobial, anti-inflammatory and osteogenic potential. Due to its high alkalinity (12.5), it has a high antibacterial activity against most bacteria found in endodontic infection. It has a destructive effect over bacteria's cellular membrane and protein structure.<sup>14</sup>

Antibacterial activity of calcium hydroxide can be attributed to direct contact to high pH and its ability to dissociate into hydroxyl ions causing bacterial cell death.<sup>15</sup>

Abundant evidence is available in literature about the resistance of *E. faecalis* to calcium hydroxide. *E. faecalis* may colonize root canals in single infection, forming dense biofilms and evading the hydroxyl ions.<sup>12</sup> Evans et al demonstrated that the proton pump activity of *E. faecalis* offers resistance to high pH of calcium hydroxide.<sup>8</sup>

In spite of the proven resistance of *E. faecalis* to calcium hydroxide, we got best results from calcium hydroxide. Statistical analysis (one way ANOVA), comparing the three tested medicines showed significant difference in the values of zone of inhibition, calcium hydroxide shows better results at both the concentrations.

Our results indicate that curcumin, triphala and calcium hydroxide are more or less effective against *E. faecalis*, calcium hydroxide showing the maximum antibacterial effect. We conclude that curcumin and Triphala are able to eliminate the matrix and bacteria which warrants further investigation.

Microbial communities in vivo are quite resistant and difficult to eradicate with antimicrobials owing to the fact that the microorganisms to be targeted are organized in structures attached to each other and/or the root canal walls, known as microbial biofilms. The testing of antimicrobial agents against bacterial biofilms is yet to be standardized and no in vitro method accurately reflects the conditions under which microorganisms grow in vivo. So, caution should be exercised while extrapolating these results to the clinical scenario.

## Conclusion:

Within the limitations of this in vitro study, we can conclude:

1. Antibacterial activity was observed in all the tested medicaments.
2. Curcumin exhibited least antibacterial activity as compared with triphala and calcium hydroxide.
3. Calcium hydroxide exhibited highest zone of inhibition in 24 hrs.
4. There was statistically significant difference in the values of zone of inhibition of calcium hydroxide, Triphala and curcumin.

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