



## COMPARATIVE ASSESSMENT OF ANTIMICROBIAL EFFICACY OF BROMELAIN, ROSEMARY EXTRACT, CALCIUM HYDROXIDE AND TAP AGAINST E. FAECALIS - AN IN VITRO STUDY

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### ABSTRACT

**Background-** Synthetic intracanal medicament like calcium hydroxide & triple antibiotic paste are the medicaments of choice for years. Synthetic intracanal medicament can be cytotoxic. So, the herbal plant derived pineapple extract like bromelain & rosemary extract can be the potential alternative with least cytotoxicity can be used as potential intracanal medicaments. **Aim:** This study aims to compare & evaluate antimicrobial efficacy of Bromelain, Rosemary extract, Calcium hydroxide & Triple antibiotic paste used as intracanal medicament against enterococcus faecalis. **Methodology:** Samples divided into four groups Group A: Calcium Hydroxide Group B: Triple Antibiotic Paste Group C: Bromelain Group D: Rosemary Extract. E. faecalis suspension inoculated on the agar plates. Intracanal medicament placed into agar plate wells and after incubation of agar plates inhibition zones measured and results derived. **Statistical Analysis:** Results were analyzed using One-way ANOVA and Post hoc Tukey test. **Results:** Mean inhibition zones for Group A, Group B, and Group C and Group D - are 18.20, 20.33, 28.87 and 11.27 respectively, with highest inhibition zones for group C Bromelain extract. **Conclusion:** Bromelain and Rosemary extract shows the promising results as the Intracanal medicaments. Considering the adverse effect of synthetic intracanal medicaments, bromelain & rosemary extract can be the potential replacement as intracanal medicaments.

**KEYWORDS :** Bromelain, Calcium Hydroxide, Enterococcus Faecalis, Intracanal Medicaments, Microorganisms, Rosemary Extract, Triple Antibiotic Paste.

### INTRODUCTION

Microorganisms and toxic byproducts are main cause of pulp and periapical diseases and also for failure of the treated cases.<sup>1</sup> The success of endodontic treatment depends on the elimination of the microorganisms and prevention of reinfection using various irrigants, activation techniques, and use of intracanal medicaments.<sup>2</sup> Interappointment bacterial growth can be reduced by placing an appropriate intracanal medicament dressing.<sup>3</sup> Intracanal medication destroys residual microorganisms, toxins and residual bacteria that are failed from removal via chemo-mechanical preparation of root canal system.<sup>4</sup>

E. faecalis is a gram positive facultative anaerobic bacterium has the ability to exist without oxygen in the changing environment and also have the ability to deeply penetrate into the dentinal tubules.<sup>5</sup> E. faecalis stay even after thorough disinfection and cause endodontic treatment failures. Among all the reported cases with pain and infection following endodontic therapy, it has been observed that E. faecalis is the most commonly found, with high prevalence values reaching up to 90%. E. Faecalis has been found in 38% of the failed root canal-treated teeth.<sup>6</sup>

Ideal intracanal medicaments should provide continuous and extended antimicrobial effect, stability in the solution, should be biocompatible, anti-bacterial, anti-fungal, and should not stain the tooth. However, till date, there is no such ideal Intracanal Medicament available.<sup>7</sup> Calcium hydroxide is the intracanal medicament of choice for many years, considered as gold standard owing to its antimicrobial properties. Calcium hydroxide has been proved to compromise the strength of the RC (root canal) system.<sup>8</sup> Triple antibiotic paste (TAP) consists of metronidazole, ciprofloxacin and minocycline, has been effectively utilized to disinfect the

canals.<sup>9</sup> Triple antibiotic paste (TAP) which was also more likely found to be effective than Calcium hydroxide and chlorhexidine gel caused bacterial resistance and tooth discoloration.<sup>10,11</sup>

Use of synthetic intracanal medicaments Calcium hydroxide & triple antibiotic paste still concerns over adverse effect & cytotoxicity.<sup>12</sup> Plant extracts and their components possessing antimicrobial properties are used in medical treatments. Herbal agents such as Propolis, Triphala, Camelliasinensis (Chai - Tea), Curcuma longa Linn. (Haridra -Turmeric), orange oil, guava, and neem with potent antimicrobial properties have been previously used to disinfect the canals.<sup>13</sup> And considered safer option than synthetic intracanal medicaments.

Bromelain is a proteolytic enzyme derived from the stem part and fruit of pineapples (ananas comosus). Bromelain is known for its proteolytic, antibacterial, antifungal, anti-inflammatory, antithrombotic, and fibrinolytic properties. Its rich content of flavonoids and protease contributes to its antibacterial properties. Its use is underexplored in the field of endodontics.<sup>14</sup>

Rosmarinus officinalis L. (Lamiaceae) is an edible evergreen shrub that possesses antioxidant, anti-inflammatory, anticancer, and antimicrobial properties for medicinal and culinary purposes. Research states that the hydroalcoholic extract of the leaves of Rosmarinus officinalis can be used in endodontic practice because of its bactericidal effect that can inhibit the growth of Enterococcus faecalis.<sup>15</sup> Considering the quantum of efficacy, the harmful effects and safety concerns of synthetic agents; the replacement with herbal agents might prove to be advantageous.

Agar diffusion test is the most widely used test to check the

antimicrobial effectiveness of the material.<sup>16</sup>Therefore the aim of the study is to use agar diffusion test to evaluate antimicrobial effectiveness of Bromelain, rosemary extract calcium hydroxide and triple antibiotic paste, as intracanal medicament against *E. faecalis*.

**MATERIALS AND METHODS**

**Experimental Intracanal Medicaments**

Triple antibiotic paste (1:1:1) – Ciprofloxacin (Ciplox 500 mg tablet, Cipla India), Metronidazole (Metrogyl 400 mg tablet, JB Chemicals and Pharmaceuticals) and minocycline (Minoz, 100 mg tablet, Ranbaxy India) Each of these powders were prepared separately by removing the enteric coatings of the tablet and crushing it to a fine powder using sterile mortar and pestle. The three antibiotic powders were weighed individually using a digital weighing machine to obtain a desired 1:1:1 proportion. The powders were mixed to obtain Triple antibiotic powder. One mg powder was mixed with one ml of sterile water to obtain a paste consistency.

Bromelain extract (1:1) - Bromelain powder with enzymatic activity of 2400 gelatin digestion unit per gram was mixed with distilled water in 1:1 proportion 1 g powder was mixed with 1 ml distilled water.

Rosemary extract – Rosemary extract powder mixed with in proportion 1 g powder was mixed with 1ml of propylene glycol.

Calcium hydroxide – PRIME dental RC Cal.

**Preparation of *E. Faecalis* Strain**

The *E. faecalis* strain was used as a test organism. It was grown on tryptone soya agar (TSB), suspended in 5 mL of TS broth and incubated for 24 h at 37 °C. The turbidity was equivalent to 0.5 McFarland standard.

Study Carried Out in 4 Groups:

- Group A: Triple antibiotic paste
- Group B: Rosemary extract
- Group C: Bromelain extract
- Group D: Calcium hydroxide

**Agar Diffusion Test**

Agar well diffusion test was performed in Petri dishes containing Nutrient Agar. Sterile cotton swab used for the inoculation of the *E. faecalis* on the nutrient agar plates. After inoculation of suspension dried at 37 °C for 15minutes.

4 Recess well prepared in each agar plate of equal diameter. A sterile syringe was used to place each medication within each plate respective well. Incubation of the samples at 37°C for 24 hrs. after incubation, inhibition zones on the agar plates measured. inhibition zones around each medicament were evidenced by the lack of bacterial colonization (clearing of agar) adjacent to each agar well the results were derived by measuring zones of inhibition.

**Statistical Analysis**

Data was analyzed using SPSS software program version 20. antibacterial effects of medicaments against *E. faecalis* and the sensitivity towards each medicament were compared using the One-way ANOVA and Tukey's post hoc tests. P value < 0.05 was considered statistically significant.

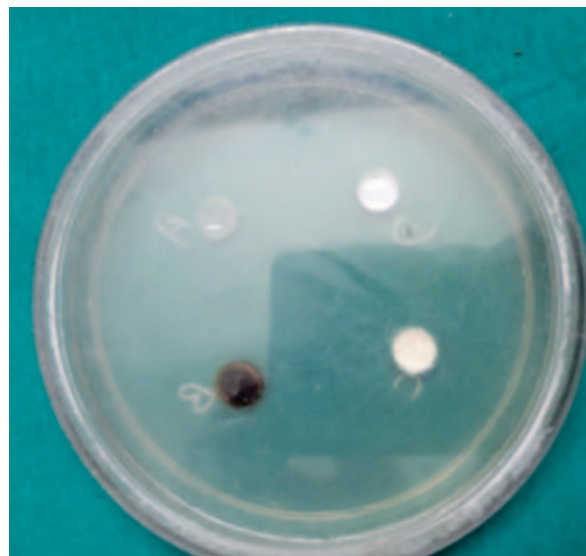
**RESULTS**

**Table 1 – Comparison of Zone of Inhibition Among Four Groups**

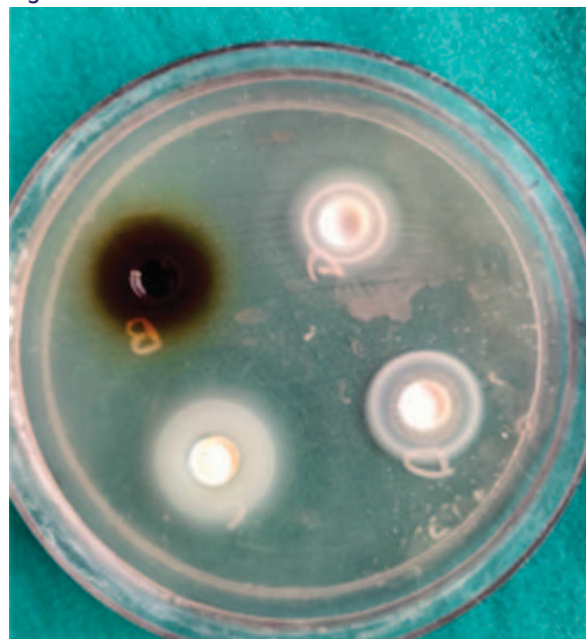
| Group   | N  | Mean  | Sd   | P value |
|---------|----|-------|------|---------|
| Group A | 15 | 18.20 | 0.68 | <0.001* |
| Group B | 15 | 20.33 | 1.11 |         |
| Group C | 15 | 28.87 | 0.99 |         |
| Group D | 15 | 11.27 | 0.80 |         |

**Table 2: Tukey's Post-hoc Analysis Showing Intergroup Comparison of Inhibition Zones**

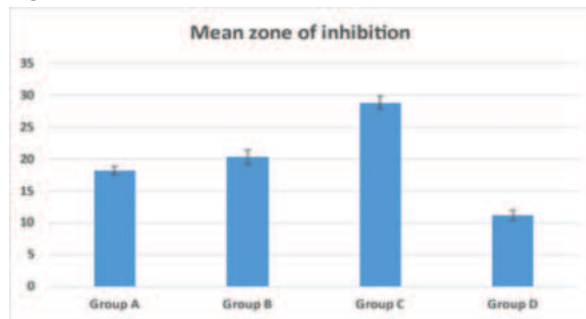
| Group pair         | P value |
|--------------------|---------|
| Group A vs Group B | <0.001* |
| Group A vs Group C | <0.001* |
| Group A vs Group D | <0.001* |
| Group B vs Group C | <0.001* |
| Group B vs Group D | <0.001* |
| Group C vs Group D | <0.001* |



**Figure 1: Inhibition Zone Before Incubation**



**Figure 2: Inhibition Zone After Incubation**



**Graph 1: Mean Inhibition Zones of the Medicaments**

The mean diameters of inhibition zones developed against the *E. faecalis* by the medicaments are shown in Table 1, Graph 1 and figure 2. Results of the one-way ANOVA test showed significant differences between the mean values of inhibition zones diameters produced by the tested medicaments ( $P < 0.05$ ). Zone of inhibition was maximum 28.87 in bromelain followed by 20.33 rosemary extract, 18.20 in TAP and 11.27 in calcium hydroxide. Difference in zone of inhibition among four groups was significant. Bromelain showed significant highest zone of inhibition as compared to other three groups. Calcium hydroxide showed lowest zone of inhibition against *E. faecalis*.

## DISCUSSION

*E. Faecalis* is the main pathogen responsible for endodontic treatment failure. Its ability to resist different disinfection measure due to its virulence & survival factor makes it difficult to eradicate from root canal system therefore *E. Faecalis* is used as the benchmark bacteria against which intracanal medicaments were tested & evaluated the antimicrobial properties of the medicaments against *e. Faecalis*.

Agar diffusion test used to access antimicrobial activity of the medicaments as it is simple method & it allows direct comparisons of the antimicrobial activity of endodontic materials by measuring the size of formed inhibition zones. Initial assessment of antimicrobial activity can be done before carrying out more advanced tests. However, the size of the inhibition zones does not indicate the entire antimicrobial efficiency of the material as results may be influenced by factors such as the chemical and physical properties of the tested material and culture medium. In current study, all attempts were made to decrease most of the variables such as type and thickness of agar medium, inoculums density, and incubation temperature.

Bromelain possess antibacterial properties attributed to its chemical compounds including saponins, tannins, flavonoids, and various enzymes. Flavonoids, in particular, possess the inherent ability to form complex bonds with extracellular proteins through hydrogen bonding, consequently altering cell membrane permeability. Results of this study shows that application of bromelain as an intracanal medicament has significantly higher inhibition zone followed by rosemary extract followed by triple antibiotic paste and then calcium hydroxide and bromelain possess potent antibacterial efficacy. The results were similar with Neelam chandwani et al. (2022) where they concluded that antimicrobial efficacy of bromelain was found to be more effective than calcium hydroxide & TAP against *E. faecalis*.<sup>17</sup> Arsyada et al. (2018) evaluated the antimicrobial efficacy of bromelain extract mixed with calcium hydroxide suggested improved antimicrobial efficacy of calcium hydroxide against *E. faecalis*. Also, bromelain has found to have higher efficacy against gram positive bacteria than gram negative.<sup>18</sup>

Rosemary extract contains bioactive compound such as phenolic diterpenes, triterpenes phenolic acid and flavonoids this compound has antibacterial properties to inhibit bacterial growth by interacting with cell membrane causing changes in genetic material and altering the transport of electrons leakage of cellular components and changes in fatty acids. Also due to interaction with protein membrane which cause loss of structure & functionality of the cells. Mirza Aryanto et al (2023) reported that rosemary extract with 5 % and 10 % concentration had inhibition of *E. faecalis*.<sup>19</sup> The results of study are consistent with study by Ashwini Dessai et al. (2022) stating that the Carnosic acid (rosemary extract) showed better antimicrobial efficacy against *E. Faecalis*.<sup>20</sup>

Triple antibiotic paste (TAP) has a broad-spectrum activity as it possesses properties of three antibiotics Ciprofloxacin,

Metronidazole and Minocycline. TAP is effective in disinfection & overcomes the resistance of poly microbial infection within the root canal system by its activity against anaerobes, aerobes, actinomycetes and other bacteria. When compared with Calcium Hydroxide (CH), TAP has twice the depth of penetration in dentine and bacterial destruction, so is recommended as a root canal disinfectant in re-treatment cases as well. TAP possess better antimicrobial activity than calcium hydroxide.<sup>21</sup>

Calcium hydroxide is most commonly used intracanal medicament. It has PH of 12.5. calcium hydroxide exhibits its antimicrobial activity by releasing hydroxyl ions ( $OH^-$ ), thereby creating a nonconductive alkaline environment for the microorganisms to survive. However, the rate of hydroxyl ion diffusion is prolonged due to the buffering capacity of the dentin, the medicament is neutralized by the bacterial products, some bacterial species have been discovered that bacteria, including *Candida* and opportunistic infection-causing pathogens like *E. faecalis*, are very resistant to calcium hydroxide.<sup>22</sup> Therefore, antimicrobial activity of calcium hydroxide is least. The results from present study showed TAP was superior than calcium hydroxide as intracanal medicament against *E. faecalis* and the results were consistent with Mozayeni et al. (2014).<sup>6</sup>

## CONCLUSION

Within limitation of study, it can be concluded that Bromelain has the highest antimicrobial potential against *E. faecalis* as the Intracanal medicament. Rosemary extract shows the promising results as the Intracanal medicament. Considering the adverse effect of synthetic intracanal medicament, bromelain & rosemary extract can be the potential replacement as intracanal medicament.

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