



**AN IN VITRO COMPARISON OF ANTIMICROBIAL EFFICACY OF TRIPLE ANTIBIOTIC PASTE, CALCIUM HYDROXIDE AND ACACIA NILOTICA (BABBULA) AGAINST ENTEROCOCCUS FAECALIS, STREPTOCOCCUS MUTANS AND CANDIDA ALBICANS AS INTRACANAL MEDICAMENTS.**

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### ABSTRACT

**Aim:** The aim of this study was to compare the antimicrobial efficacy of Triple antibiotic paste (TAP), Calcium hydroxide and *Acacia nilotica* (*A. nilotica*) (Babbula) against *Enterococcus faecalis* (*E. faecalis*), *Streptococcus mutans* (*S. mutans*) and *Candida albicans* (*C. albicans*) as intracanal medicaments.

**Materials and methods:** The triple antibiotic paste mixture of (metronidazole, ciprofloxacin and minocycline), calcium hydroxide, *Acacia nilotica* (Babbula) were weighed and mixed with polyethylene glycol and agar well diffusion method was employed to study the antimicrobial efficiency against *Enterococcus faecalis*, *Streptococcus mutans* and *Candida albicans*

**Results:** All the intracanal medicaments showed zone of inhibition against *Enterococcus faecalis*, *Streptococcus mutans* and *Candida albicans*. Among all intracanal medicaments used in the study Triple antibiotic paste produced maximum zone of inhibition followed by Babbula and least by Calcium hydroxide.

**Conclusions:** Triple antibiotic paste showed the best antimicrobial efficacy followed by Babbula and least by Calcium hydroxide against *Enterococcus faecalis*, *Streptococcus mutans* and *Candida albicans*. More phytochemical studies need to be done. Further evaluation of concentrated extracts of *Acacia nilotica* (Babbula) could yield better results.

**KEYWORDS :** Triple antibiotic paste, calcium hydroxide, *Acacia nilotica*, intracanal medicaments.

### INTRODUCTION

The main aim of root canal treatment is to completely eradicate the microorganisms from the root canal system. However, the root canal configuration is complex and the bacteria within the canal cannot be completely debrided with mechanical instrumentation alone.<sup>(1)</sup> Hence, intracanal medicaments are used along with instrumentation in aiding the disinfection of the root canal system.<sup>(2)</sup>

*Enterococcus faecalis* microorganism is notoriously famous and has been isolated in about 24%–77% cases of failure of root canal therapy. This might be due to the ability of this microorganism to withstand high alkaline environment. It survives deep in the dentinal tubules as biofilm and colonizes the root canal.<sup>(3)</sup> *Candida albicans* is another microorganism commonly isolated from failed root canals. It breaks down collagen in dentinal wall for its nutrient supply and colonizes inside the root canal.<sup>(4)</sup> *Streptococcus mutans* is a gram-positive cocci that are usually found in the mouth, and plays a role in formation of dental caries. The bacterium has a polysaccharide coat (glycocalyx) that allows it to stick to the teeth. It also produces acid from sugar in saliva, and this promotes erosion of tooth enamel.<sup>(5)</sup> *S. mutans* invades toward pulp and its presence was elucidated in inflamed root canals.<sup>(6)</sup>

Biomechanical preparation along with chemical agent of root canal reduces the bacterial load but use of intracanal medicaments and its action against bacterial agents is required to increase the sterility /decontamination of root canal.<sup>(7)</sup>

The benchmark of intracanal medicaments is calcium hydroxide and remains popular till date.<sup>(8)</sup> It is commonly used as an inter-appointment intracanal dressing due to its good biocompatibility.<sup>(9)</sup> It has wide range of antibacterial activity against the pathogens of the root canal. But it has been found less effective against *E. faecalis* as it has been shown to resist high alkaline pH during calcium hydroxide dressing.<sup>(10)</sup> Triple antibiotic paste (TAP), is used which is a combination of

ciprofloxacin, metronidazole and minocycline has been proven to be effective intracanal medicament. But, minocycline in TAP cause discoloration.<sup>(11)</sup>

Herbal agents have been used in dentistry which are since ancient times as phytomedicines. The medicinal properties of natural herbal products are useful and have been effective against various diseases. *Acacia nilotica* commonly known as Gum Arabic tree and Babul in India is a multipurpose tree. It is widely spread in subtropical and tropical Africa to the middle east and in Asia Pakistan and India. The plant has bright yellow coloured flowers and long grey constricted pods.<sup>(12)</sup> The use of *Acacia nilotica* in treatment of a cold, cough, diarrhoea, dysentery, jaundice and skin disorders have been proven.<sup>(13-16)</sup>

The present study aimed to compare the antimicrobial efficacy of Triple antibiotic paste, Calcium hydroxide and *Acacia nilotica* (Babbula) as intracanal medicaments against *Enterococcus faecalis*, *Streptococcus mutans* and *Candida albicans* species.

### MATERIALS AND METHODS

#### MATERIALS

Preparation of Triple antibiotic paste – Equal amounts metronidazole, ciprofloxacin and minocycline tablets were crushed in 1:1:1 ratio. This was made in powdered form and weight of 50 mg and 100 mg mixed in with polyethylene glycol to make it in paste form.

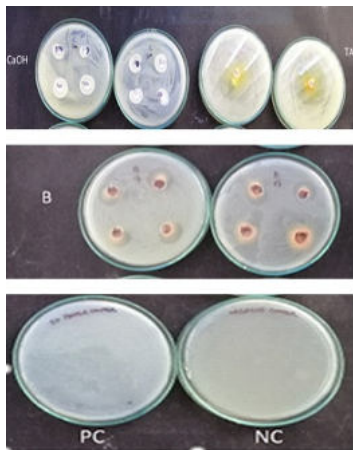
Preparation of Calcium hydroxide paste – 50 mg and 100 mg of calcium hydroxide powder were weighed separately and made into paste with polyethylene glycol, inoculated and incubated at 37°C for 24 hours.

Preparation of Babbula paste –The bark of Babbula tree (*Acacia nilotica*) was dried, crushed and made in powdered form. The powder was weighed as 50 mg and 100 mg and mixed it with polyethylene glycol to form a paste.

**METHODS:**

Standard strains of *Enterococcus faecalis*, *Candida albicans* and *Streptococcus mutans* (ATCC 29212) were collected from Rajarajeswari Medical College & Hospital, Bengaluru. It was cultivated in nutrient broth and incubated at 37° C for 24 hours, checked for growth under spectrophotometry. Wells of about 6 mm diameter were made and the microorganisms were spread on the culture plate. Triple antibiotic paste, calcium hydroxide paste, Babbula paste and normal saline (control) were added to respective wells and plates and incubated for 24 hours at 37° C in an incubator. The activity of control group was checked for any zone of inhibition against culture. After incubation period, the plates were checked for zones of inhibition and was recorded in millimeter. The study was performed three times and mean zone of inhibition was recorded in millimeter.

**RESULTS**

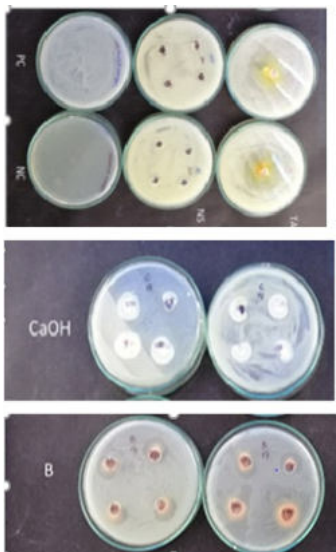


**Fig 1: Antimicrobial activity against *Enterococcus faecalis***

\*Note: CaOH-Calcium hydroxide, B-Babbula, TA-Triple antibiotic, NS-Normal saline, PC-Positive control, NC-Negative control

**Table 1: Fig: Antimicrobial activity against *Enterococcus faecalis***

Sample	Diameter of zone of inhibition in mm	
	50mg	100mg
Calcium Hydroxide	15.2	15.1
Babbula	17.8	20.1
Triple antibiotic paste	43.0	47.65

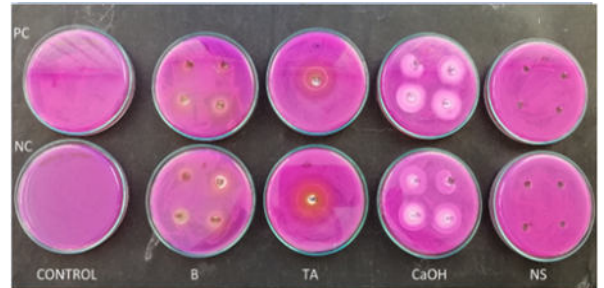


**Fig 2: Antimicrobial activity against *Streptococcus mutans***

\*Note: CaOH-Calcium hydroxide, B-Babbula, TA-Triple antibiotic, NS-Normal saline, PC-Positive control, NC-negative control

**Table 2: Antimicrobial activity against *Streptococcus mutans***

Sample	Diameter of zone of inhibition in mm	
	50mg	100mg
Calcium Hydroxide	12.2	15.0
Babbula	19.2	19.0
Triple antibiotic paste	44.6	50.2



**Fig 3: Antimicrobial activity against *Candida albicans***

\*Note: CaOH-Calcium hydroxide, B-Babbula, TA-Triple antibiotic, NS-Normal saline, PC-Positive control, NC-negative control

**Table 3: Antimicrobial activity against *Candida albicans***

Sample	Diameter of zone of inhibition in mm	
	50mg	100mg
Calcium Hydroxide	11.8	20.2
Babbula	15.1	17.05
Triple antibiotic paste	35.1	45.05

**STATISTICAL ANALYSIS**

The results were statistically analysed by one-way ANOVA Test and Tukey's post hoc Analysis test.

Comparison of mean Zone of Inhibition (in mm) for E. Faecalis between Calcium hydroxide, Babbula & TAP at 50 & 100 mg concentrations using One-way ANOVA Test followed by Tukey's Post hoc Analysis										
Conc.	Ca(OH) <sup>2</sup>		Babbula		TAP		P-Value	Tukey's HSD Post hoc Test		
	Mean	SD	Mean	SD	Mean	SD		C Vs B	C Vs T	B Vs T
50 mg	15.20	0.20	17.97	0.25	43.07	0.12	<0.001*	<0.001*	<0.001*	<0.001*
100 mg	15.17	0.15	20.17	0.15	47.77	0.25	<0.001*	0.02*	<0.001*	<0.001*

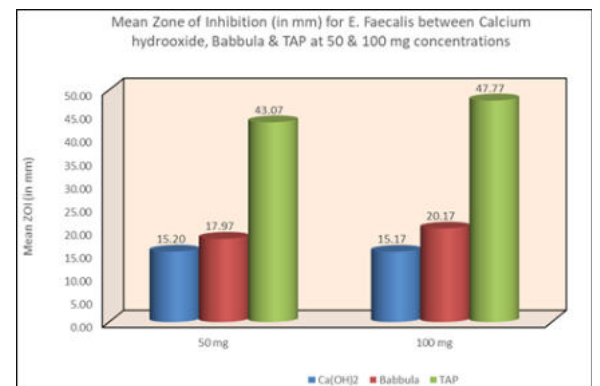
Comparison of mean Zone of Inhibition (in mm) for S. Mutans between Calcium hydroxide, Babbula & TAP at 50 & 100 mg concentrations using One-way ANOVA Test followed by Tukey's Post hoc Analysis										
Conc.	Ca(OH) <sup>2</sup>		Babbula		TAP		P-Value	Tukey's HSD Post hoc Test		
	Mean	SD	Mean	SD	Mean	SD		C Vs B	C Vs T	B Vs T
50 mg	12.23	0.21	19.33	0.31	44.67	0.15	<0.001*	<0.001*	<0.001*	<0.001*
100 mg	15.07	0.12	19.07	0.12	50.20	0.20	<0.001*	<0.001*	<0.001*	<0.001*

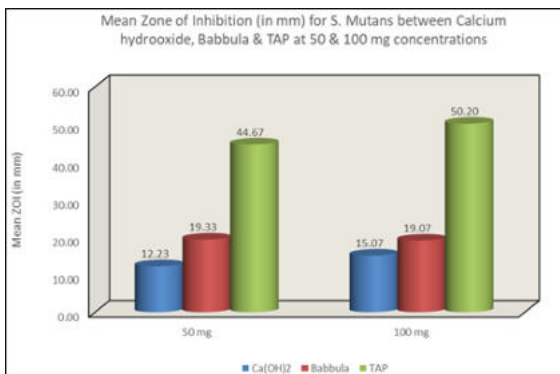
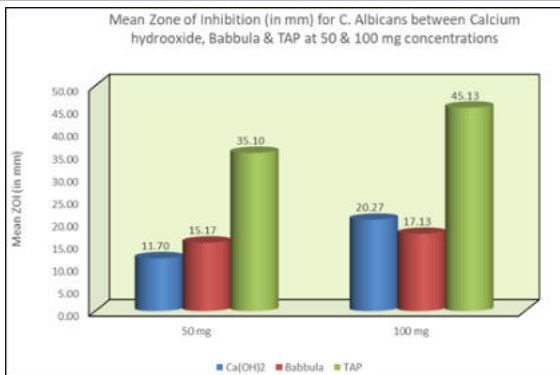
  

Comparison of mean Zone of Inhibition (in mm) for C. Albicans between Calcium hydroxide, Babbula & TAP at 50 & 100 mg concentrations using One-way ANOVA Test followed by Tukey's Post hoc Analysis										
Conc.	Ca(OH) <sup>2</sup>		Babbula		TAP		P-Value	Tukey's HSD Post hoc Test		
	Mean	SD	Mean	SD	Mean	SD		C Vs B	C Vs T	B Vs T
50 mg	11.70	0.30	15.17	0.15	35.10	0.10	<0.001*	<0.001*	<0.001*	<0.001*
100 mg	20.27	0.23	17.13	0.15	45.13	0.15	<0.001*	<0.001*	<0.001*	<0.001*

\*Statistically significant

Note: C- Ca(OH)<sub>2</sub>, B- Babbula, T- TAP





## DISCUSSION

The goal of root canal treatment is to eliminate the primary etiologic agent of endodontic disease i.e. bacteria from the root canal. The microorganisms in root canal are polymicrobial in nature consisting of anaerobic and facultative anaerobic microorganisms. The microorganisms *Enterococcus faecalis* and *Candida albicans* are frequently isolated in cases of failed root canal treatment.<sup>(17)</sup> In cases of failed root-filled teeth which is associated with apical periodontitis, the most frequently isolated organisms are *Enterococcus faecalis* and *Candida albicans* are considered to be the most defiance microorganisms in endodontic infections and are implicated as an accessible cause of root canal treatment failure.<sup>(18)</sup> Both microbes have been detached from infected root canal.<sup>(20-24)</sup> However, *Enterococcus* species are shown to be capable of withstanding high pH changes as well as prolonged periods even with little nutrient supply.<sup>(25)</sup>

Calcium hydroxide is considered the benchmark of intracanal medicaments. It releases hydroxyl ions in a fluidic environment which is responsible for its antimicrobial action. The hydroxyl ions causes damage to the cytoplasm of the bacteria leading to protein denaturation and damage to bacterial DNA.<sup>(26)</sup>

Triple antibiotic paste is a mixture consisting of ciprofloxacin, metronidazole and minocycline.<sup>(27)</sup> Metronidazole has antibacterial action against anaerobic bacteria. Minocycline has bacteriostatic action on gram-positive and gram-negative bacteria. It also causes an increase in the amount of interleukin-10, which is an inflammatory cytokine. Ciprofloxacin has limited action against anaerobic bacteria while the most susceptible one are the aerobic gram negative bacilli. It acts through the inhibition of DNA gyrase, resulting in degradation of the DNA by exonucleases. It is with metronidazole in the treatment of aerobic and anaerobic infections.<sup>(28)</sup> Therefore, TAP is effective against wide range of microorganisms i.e. gram-positive, gram-negative and anaerobic bacteria, present in odontogenic infections.<sup>(29)</sup>

Natural products are sometimes considered as alternatives medicines and have been used in India as Ayurveda dating

back several thousand years.<sup>(30)</sup> The World Health Organization (WHO) observed that a great number of herbal extracts are being used in modern medicine. The defence mechanisms present in plants consist of aromatic compounds such as tannins, flavonoids and alkaloids. They help plants in defence against microorganisms, insects and animals in nature. These natural agents present in plants have been isolated for its beneficial properties. Flavonoids present in plants has action on to form a complex with bacterial cell wall and proteins. The antimicrobial activity of phenolic compounds have been shown to be effective in arresting the growth of *Candida albicans*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Saponins have been shown effective against bacterial and fungal pathogens (Gopishkhanna and Kannabiran, 2008).<sup>(31)</sup> In the present study, *Acacia nilotica* was used as it has been shown to possess antibacterial property. Crude alkaloids of *Acacia nilotica* leaves had higher inhibitory potential against bacterial pathogens such as *S. aureus*.<sup>(32)</sup> Deshpande et al. found similar results regarding the effective antimicrobial efficacy of *Acacia nilotica* against *S. mutans*.<sup>(33)</sup>

The zone of inhibition against *E. faecalis* for *A. nilotica* (Babbula) was 17.8 mm at 50 mg and 20.1 mm at 100 mg, calcium hydroxide showed result 15.2 mm at 50 mg and 15.1 mm at 100 mg and triple antibiotic paste showed result 43 mm at 50 mg and 47.65 mm at 100 mg respectively. The zone of inhibition against *Streptococcus mutans* was *A. nilotica* (Babbula) showed result 19.2 mm at 50 mg and 19 mm at 100 mg, calcium hydroxide showed result 12.2 mm at 50 mg and 15 mm at 100 mg, and triple antibiotic paste showed result 44.6 mm at 50 mg and 50.2 mm at 100 mg respectively. The zone of inhibition against *Candida albicans* was *A. nilotica* (Babbula) showed result 15.1 mm at 50 mg and 17.05 mm at 100 mg, calcium hydroxide showed result 11.8 mm at 50 mg and 20.2 mm at 100 mg and triple antibiotic paste showed result 35.1 mm at 50 mg and 45.05 mm at 100 mg.

Our study demonstrated that *A. nilotica* (Babbula) has antimicrobial activity against *E. faecalis*, *S. mutans* and *C. albicans* but its potency is better compared to Calcium hydroxide but is less compared to medicaments triple antibiotic paste.

In the present study, Triple antibiotic paste showed maximum zone of inhibition, followed by Babbula and least Calcium hydroxide at 50 mg and 100 mg concentration against *E. faecalis*, *S. mutans* and *C. albicans*. Overall, the results of our study showed that triple antibiotic paste was most effective with highest zone of inhibition against *E. faecalis*, *S. mutans* and *C. albicans*.

## CONCLUSION:

Triple antibiotic paste showed the best antimicrobial efficacy followed by Babbula and least by Calcium hydroxide against *Enterococcus faecalis*, *Streptococcus mutans* and *Candida albicans*. More phytochemical studies need to be done. Further evaluation of concentrated extracts of *Acacia nilotica* (Babbula) could yield better results.

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