



ISSN 2456-3110

Vol 5 · Issue 6

Nov-Dec 2020

Journal of
**Ayurveda and Integrated
Medical Sciences**

www.jaims.in

JAIMS

An International Journal for Researches in Ayurveda and Allied Sciences



Charaka
Publications

Indexed

Antibacterial effectiveness of natural products alone and in combination with Calcium Hydroxide as Intracanal Medicaments : An In-Vitro Microbiological Study

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ABSTRACT

Major objective of root canal treatment is disinfection of the root canal system and elimination of microbiota. Various intracanal medicaments as an adjunct to mechanical disinfection and irrigation have been used but due to the harmful effects of the commercially available agents, a need for natural alternatives is felt over the time. This in-vitro study aims to evaluate the antibacterial effectiveness of natural agents Septillin and *Aloe vera* with Calcium hydroxide and without Calcium hydroxide on the *Streptococcus mitis*, *E. Faecalis* and *Candida albicans* using agar well diffusion method. The antibacterial activity was assessed by the diameter of zone of inhibition seen as clear zone around the wells. The study showed that Septillin in combination with Calcium hydroxide and alone showed considerable antimicrobial activity against *Streptococcus mitis*, *Enterococcus faecalis* and *Candida albicans* where as *Aloe vera* with Calcium hydroxide showed to be mildly effective against all three but not as effective as Septillin and Calcium hydroxide when tested alone.

Key words: Intracanal medicaments, antibacterial activity, Septillin, Calcium hydroxide.

INTRODUCTION

The primary intention of endodontic treatment is disinfecting the root canal anatomy three dimensionally. Majority of the bacterial load is reduced by mechanical and chemical means. *Streptococcus sp.*, *Fusobacterium nucleatum*, *Bacteroides sp.*, *Enterococcus faecalis*, *Candida albicans*, *Prevotella intermedia*, etc. are some of the routinely encountered microbes causing infection in the root canal system. The complex root anatomy and

resistant microbes are responsible for possible failures and the use of antimicrobial agents in the form of intracanal medicaments effectively aid in the reduction of failure possibilities and chances of re-infection. Most of the commercially available intracanal medicaments used have cytotoxic effects. Also they often fail to eliminate bacteria completely from the dentinal tubules. To overcome these shortcomings the trend to use biologic medication extracted from natural plants have come into existence. Calcium hydroxide in various forms is being traditionally used in the canal as a potent medicament. Basic pH of 12.5 being responsible for rapid elimination of bacterial substance.^[1] The action of buffers like bicarbonates, phosphates, acids, proteins and CO₂ reduce the hydroxyl ion concentration and the antibacterial effectiveness of calcium hydroxide may be reduced or impeded.^[2] Commercially available medicaments possess chemicals that might cause various side effects and also resistance to the bacterial strains in the canals, rendering the need for using natural antimicrobial

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Submission Date: 17/10/2020 Accepted Date: 23/12/2020

Access this article online

Quick Response Code



Website: www.jaims.in

DOI: 10.21760/jaims.5.6.10

agents inside the root canals. *Aloe barbadensis* Miller is a plant with excellent medicinal properties. Aloins and Barbadoins are the chief constituents.^[3] Aphthous ulcers, Lichen planus, inflammation of the alveolus are the routine dental conditions in which it is being used.^[4] *A. Streptococcus pyogenes* and *Enterococcus faecalis* are arrested with the aid of anthraquinone, one of its main constituent.^[5] Septillin is another herbal product whose antibacterial, anti-inflammatory and antiexudative properties are proved to be extremely beneficial in many infections. It contains *Commiphora Wightii*, Guduchi and Yashtimadhu as main ingredient. Taking into account all these beneficial properties of natural products, their use as intracanal medicaments with less caustic and cytotoxic effects as compared to other synthetic chemicals can be satisfactory. This study aimed in finding antibacterial effectiveness of *Aloe vera* and Septillin alone and also in combination with the gold standard medicament, Calcium hydroxide.

AIM

To evaluate and compare the antibacterial effectiveness of *Aloe vera* and Septillin with or without calcium hydroxide as intracanal medicaments.

OBJECTIVES

1. To evaluate the antibacterial efficacy of Calcium Hydroxide as intracanal medicament (Control).
2. To evaluate the antibacterial efficacy of *Aloe vera* with and without calcium hydroxide as intracanal medicament.
3. To evaluate the antibacterial efficacy of Septillin® with and without calcium hydroxide as intracanal medicament.
4. To compare the antimicrobial efficacy of all the above medicaments.

MATERIALS AND METHODS

Group A: Calcium hydroxide/Control

- Group A1: *Streptococcus mitis*

- Group A2: *Enterococcus faecalis*

- Group A3: *Candida albicans*

Group B: *Aloe vera*

- Group B1: *Streptococcus mitis*

- Group B2: *Enterococcus faecalis*

- Group B3: *Candida albicans*

Group C: *Aloe vera* + Calcium hydroxide

- Group C1: *Streptococcus mitis*

- Group C2: *Enterococcus faecalis*

- Group C3: *Candida albicans*

Group D: Septillin

- Group D1: *Streptococcus mitis*

- Group D2: *Enterococcus faecalis*

- Group D3: *Candida albicans*

Group E: Septillin + Calcium hydroxide

- Group E1: *Streptococcus mitis*

- Group E2: *Enterococcus faecalis*

- Group E3: *Candida albicans*

Methodology

Agar-Well Diffusion Technique was used to carry out the procedure. After full night culture of the strains being tested in thioglycolate broth, it was streaked on the blood agar plate.(Fig 1) Wells (6 mm × 6 mm) measure for each medicament were punched out from the surface of the agar plate with the aid of template.(Fig 2) Delivery of each medicament (approx 0.1 ml) was done using a micropipette. Sterile conditions were maintained through-out the procedure so as to avoid any contaminations. Incubation at 37°C for 24hours was carried out followed by close monitoring of clear zones around the wells of respective medicaments. Antibacterial effectiveness was assessed by measuring the diameters of the clear zones denoted as zones of inhibition.(Fig 4,5,6)

RESULTS

Medicaments	<i>Streptococcus mitis</i>	<i>E. faecalis</i>	<i>Candida albicans</i>
Group A (CH)	A1= 16 mm	A2= 10 mm	A3= 14 mm
Group B (AV)	B1=7 mm	B2=0 mm	B3=0 mm
Group C (AV + CH)	C1=11 mm	C2= 10 mm	C3= 8 mm
Group D (S)	D1= 24 mm	D2= 10 mm	D3=0 mm
Group E (S + CH)	E1= 30 mm	E2=16 mm	E3= 14 mm

DISCUSSION

Various microbial species are resistant to the commercially available medicaments. *Enterococcus faecalis*, an anaerobic gram-positive coccus is found to be persistently present inside and surrounding the canals despite the action of strong disinfectants.^[6] *Candida albicans* (CA) being the most frequently found fungus in endodontic treatment failures was chosen for this study whereas *Streptococcus mitis*, a facultative anaerobic gram-positive species is most predominantly found in primary endodontic infection.

In the present study, *Aloe vera* when combined with calcium hydroxide showed mild zone of inhibition against *streptococcus mitis* (11mm), *E. faecalis* (10mm) and *Candida albicans* (8mm) whereas when used alone showed mild results only for *streptococcus mitis* upto 7mm. In a similar study done by Kaveri and colleagues proved that *Aloe vera* extract is efficient against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli* as well as fungi like *Aspergillus niger* and *Candida*.^[7] Also another study by Jain S and co-workers confirmed *Aloe vera* gel and *Triphala* as a promising natural adjunct for antifungal agents.^[8]

Septillin® in our study showed the greatest zone of inhibition when used alone for *Streptococcus mitis* (24mm) as well for *E. faecalis* (10mm) but was ineffective against *Candida*. Greatest zone of inhibition was also observed when Septillin was used

along with calcium hydroxide on *streptococcus mitis* (30mm), *E. faecalis* (16mm) as well as in *Candida* (14mm). Contrary to which a study by Khandelwal et al.(2020), Septillin showed less anti-microbial activity compared to calcium hydroxide and chlorhexidine against *E. faecalis* and *Streptococcus mutans*.^[9] Where as a study by Priya S et al.³⁰ stated that 2% chlorhexidine and Septillin demonstrated significant inhibition followed by *Aloe vera*, Propolis and Calcium Hydroxide on *E. faecalis*.^[10]

Mozayeni et al. had conducted a study to examine the activity of various medicaments on *Enterococcus Faecalis* and concluded Calcium Hydroxide to have no considerable effect on *E. faecalis*.^[11] Contrary to which our study showed positive results with calcium hydroxide on all the three microorganisms.



Fig. 1



Fig. 2

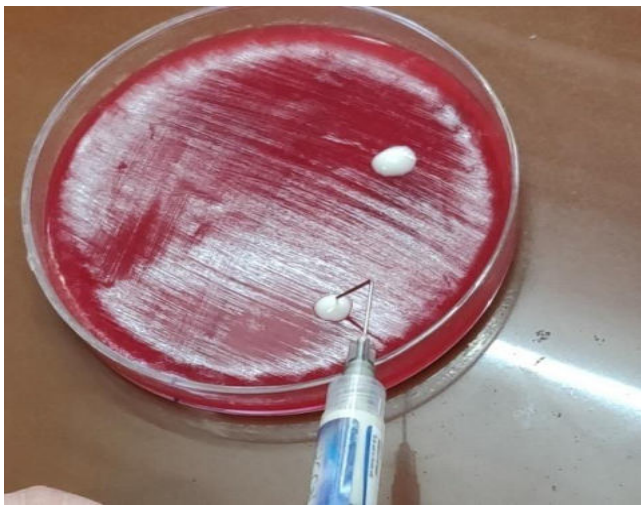


Fig. 3



Fig. 6



Fig. 4



Fig. 5

CONCLUSION

Septilin alone or in combination with calcium hydroxide is an effective and hence, a promising intracanal medicament. *Aloe vera* in combination with calcium hydroxide has mild antibacterial efficacy.

LIMITATIONS

Small sample size was taken in this in-vitro study. Hence more clinical studies are needed to substantiate the positive results obtained from this study.

ACKNOWLEDGEMENT

We sincerely acknowledge Dr. Rabindra N. Mishra Professor & HOD, Dr. Shahzad Beg Mirza Asst. Professor, Dept. of Microbiology, Dr. D.Y. Patil Medical College and Hospital, Pimpri, Pune, for their valuable guidance and support.

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How to cite this article: Dr. Abhilasha Bhawalkar, Dr. Shreya Gulati, Dr. Sanjyot Mulay, Dr. Rajesh Shetty. Antibacterial effectiveness of natural products alone and in combination with Calcium Hydroxide as Intracanal Medicaments : An In-Vitro Microbiological Study. J Ayurveda Integr Med Sci 2020;6:82-86. <http://dx.doi.org/10.21760/jaims.5.6.10>

Source of Support: Nil, **Conflict of Interest:** None declared.

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