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Comparative Evaluation of Antimicrobial Efficacy of Herbal Vs Non-Herbal Intracanal Medicaments Against Enterococcus Faecalis: An Invitro Study

Dr. Yuvadharini P[1], Dr. Vinodh S[2], Dr. Manoharan M[3], Dr. Kamatchi M[4], Dr. Karthipriya G[5]*, Dr. Kowsalya S[6]

[1]Postgraduate, [2]Professor & Head, [3]Professor, [4]Reader, Department of Pediatric and Preventive Dentistry, Vivekanandha Dental College for Women, Tiruchengode, Namakkal district – 637205, Tamil Nadu, India.

[5]Department of Conservative dentistry & Endodontics, Vivekanandha Dental College for Women, Tiruchengode, Namakkal district – 637205, Tamil Nadu, India.

[6]Department of Pediatric and Preventive Dentistry, Priyadharshini Dental College & hospital, V.G.R. Nagar, Pandur, Thiruvallur district - 631203, Tamil Nadu, India

*Corresponding author: Dr. Karthipriya G, Department of Conservative dentistry & Endodontics, Vivekanandha Dental College for Women, Tiruchengode, Namakkal dt – 637205.

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KEYWORDS

Antimicrobial efficacy

Dental caries

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Zone of inhibition

ABSTRACT:

Introduction: Dental caries is the most widespread disease affecting humans and stands as the leading chronic condition in children worldwide. Intracanal medication is often recommended for treating infected root canals to eliminate the bacteria, reduce inflammation, sterilize the canal, break down tissue debris, and provide a protective seal & to dry persistently wet canals.

Aim: The aim of the study was to evaluate & compare the antimicrobial efficacy of two herbal vs commonly used intracanal medicaments against Enterococcus faecalis by agar diffusion method.

Methods and Material: Brain Heart Infusion (BHI) prepared. Each BHI agar plate was flooded with E.faecalis and incubated at 37°C for 24 hours. Each plate of agar was allocated with five group of medicaments, Group 1: Acalypha Indica paste, Group 2: Euphorbia Hirta paste, Group 3: Triple antibiotic paste, Group 4: Chlorhexidine gluconate gel, Group 5: Calcium hydroxide paste. 5 wells were created in each agar plate and filled with medicaments. Then the plates were incubated at 37°C for 72 hours. Microbial zone of inhibition was measured & mean value was calculated.

Result: Data were analysed using one-way ANOVA and Tukey's HSD post hoc test.At 72 hours incubation period, the microbial zone of inhibition was higher in Group 3 followed by Group 2, Group 1, Group 5 and Group 1. A statistically significant difference was observed among each group.

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Conclusion: Triple antibiotic paste shows the highest antimicrobial activity against E. Faecalis followed by Euphorbia Hirta. Chlorhexidine gel shows the least antimicrobial activity among these groups of medicaments.

1. Introduction

A remarkably extensive assemblage of microorganisms resides within the intricate ecosystem of the oral cavity, which serves as a dynamic habitat for various microbial species. The occurrence of root canal infections typically transpires subsequent to the necrosis of dental pulp, a pathological condition that arises as a sequelae stemming from both dental caries and traumatic injuries to the tooth structure.[1] These intra-radicular infections are predominantly instigated by a diverse array of microorganisms, including but not limited to E. faecalis, various species of Prevotella, species of Porphyromonas, species of Fusobacterium, as well as a range of Candida species, among numerous other microbial entities.[2] Moreover, it is important to highlight that facultative anaerobes represent a particular category of microorganisms that possess the remarkable capability to thrive in both aerobic and anaerobic environments, thereby demonstrating their versatility in adapting to varying oxygen conditions.[1] One such facultative anaerobe, E.faecalis (Enterococcus faecalis), is frequently identified in clinical scenarios characterized by persistent intra-radicular infections, particularly in cases involving teeth that have undergone failed endodontic treatments.[3] The implementation of irrigation techniques is anticipated to significantly diminish the population of microorganisms present within the canal system; however, it is crucial to note that the efficacy of this process will be markedly enhanced when complemented by the strategic application of medicaments, which plays an essential role in achieving optimal outcomes. Plethora of empirical research studies has extensively investigated the comparative effects of several intracanal medicaments, such as triple antibiotic paste (TAP), calcium hydroxide (CaOH), and chlorhexidine gluconate (CHX), all of which have been recognized and established as the benchmark or gold standard in endodontic therapy.[4,5] Recently more researchers are interested in evaluating different herbal extracts for their antimicrobial efficacy, low cost & less side effects.[6]

2. Objectives

Herbal medications like aloe vera, curcuma longa (turmeric), Azadiratcha Indica (neem), propolis etc., are used as pulp dentin repair material, cleaning and disinfecting agent, sealant, smear layer removal and storage media for avulsed tooth.[7] According to Jannat et al and Govindarajan et al, Acalypha Indica (A.indica) is known to be a good antibacterial, analgesic and anti-inflammatory agent.[8,9] Rajeh et al stated that Euphorbia Hirta (E.hirta) can be used as an antibacterial and anticandidal agent.[10] Since there is paucity of information on A.indica & E.hirta as intracanal medicament, in our present study we aimed to evaluate and compare these herbal medicaments with the non-herbal medicaments. This study uses the agar diffusion method to assess and compare the antibacterial efficiency of A.indica, E.hirta, TAP, CaOH and CHX as intracanal medications against Enterococcus faecalis.

3. Methods

Ethical approval was obtained from Institutional Review Board. Followed by which, study was carried out. Sample size was calculated using G power software in which the total of 45 samples were included in this invitro study.

Brain Heart Infusion (BHI) agar prepared and poured into petridishes of 125 mm. Also, BHI broth prepared and stored in vial for two days. E.

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faecalis cultures were grown in BHI broth at 37°C for 24 hours followed by which the turbidity was checked to confirm the bacterial growth. Each agar plate's uppermost surface was flooded with 200 µl of the E. faecalis microbial strain's inoculum suspension, and it was then incubated at 37°C for 24 hours.[11]

A.indica & E.hirta leaves were collected and rinsed in tap water and shade dried for ten days. Subsequently it was ground into powder with pulverizer. Methanol & Ethanol has been used as a solvent for A.indica & E.hirta in the process of Soxhlet extraction.[9,12] In order to obtain the powder form, rotary evaporator was used to dry the extract thoroughly.

Five powder & gel extracts (Figure 1) were taken for this study.



Figure 1: Five intracanal medicaments taken in this study

Five pastes used in this study whose compositions and concentrations are as follows:

- A.indica powder and saline were mixed in concentration of 3mg/Ml.[9]
- E.hirta powder and saline were mixed in concentration of 3mg/mL.[13]
- In the ratio of 1:1:1, mixture of ciprofloxacin, metronidazole & minocycline were mixed in concentration of 16µg/mL.[14]
- CHX gel was obtained in the concentration of 1%.[11]
- CaOH powder & saline were mixed in concentration of 750µg/mL.[15]

Every agar plate features five groups of medications assigned to it,

- Group 1: A.indica paste
- Group 2: E.hirta paste
- Group 3: TAP
- Group 4: CHX gel
- Group 5: CaOH paste

In each agar plate, five wells with a seven-millimeter (mm) diameter and a four mm punch were made, and ten microlitre (µl) of medication was added. The plates were allowed to incubate for 72 hours at 37°C. Then microbial zone of inhibition (ZOI) (Figure 2) measured in millimeters with antibiotic zone scale[11] and mean value was determined.[16]

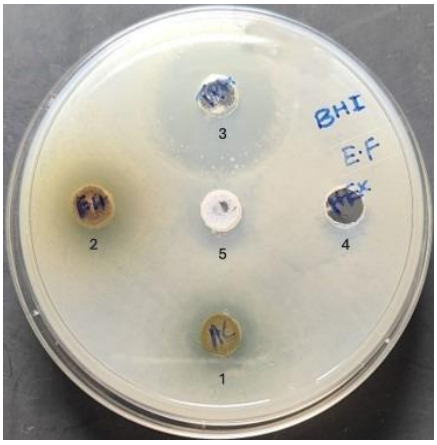

Figure 2: Zone of inhibition of all 5 groups of intracanal medicaments

4. Results

Comparison of ZOI of various intracanal medicaments shows the largest mean value in Group 3 and the lowest mean value in Group 4. Group differences were very significant ($p < 0.001$) and had a considerable effect size (Table 1).

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TABLE 1: Comparison of zone of inhibition of various intracanal medicaments

Groups	N	Mean ± SD (mm)	F	P value	Effect Size (η^2)
Group 1	45	14.9 ± 0.7	10678.5	<0.001**	0.9948
Group 2	45	20.9 ± 0.6			
Group 3	45	34.8 ± 0.7			
Group 4	45	7.3 ± 0.4			
Group 5	45	17.5 ± 0.5			

****Highly significant at p<0.001**

Multiple pairwise comparison among various intracanal medicaments envelopes the comparison between each group with the other. It shows high mean difference between TAP and all the other 4 groups. Calcium hydroxide and A.indica shows the lowest mean difference among all group comparisons (Table 2).

TABLE 2: Multiple pairwise comparison among various intracanal medicaments

Dependent variable	Reference group	Comparison group	Mean Difference (mm)
Zone of Inhibition	Group 3	Group 4	27.30**
		Group 1	19.70**
		Group 2	13.70**
		Group 5	17.20**
	Group 2	Group 4	13.60**
		Group 1	6.00**
		Group 5	3.50**
	Group 5	Group 4	10.10**
		Group 1	2.50**
	Group 1	Group 4	7.60**

****Highly significant at p<0.001**

Every pairwise comparison revealed differences that were statistically significant (p<0.001).

5. Discussion

Paramount objective for the successful execution of endodontic procedure is to eradicate every microorganism, along with their byproducts as well as pulp debris, within compromised pulp chambers, accompanied by thorough filling and decontamination of microbial presence within the canal space. The efficacy of root canal treatment hinges upon biomechanical preparation, followed by effective depuration along with obturation. The application of intracanal medicaments contributes significantly to destruction of pathogens.[17]

Relentless and ever-accelerating rise in the prevalence of antibiotic-resistant bacterial strains, coupled with the myriad adverse effects that are typically correlated with synthetic pharmaceutical drugs, has significantly motivated and spurred researchers and scientists alike to diligently seek out and explore viable herbal alternatives that may provide effective solutions to these pressing health challenges.[18] Herbal medicaments like aloe vera, curcumin, neem, honey and so on are used as pulp

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dentin repair material, sealant, smear layer removal, cleaning and disinfecting agent etc.,[7]

According to Govindarajan et al., (2008), leaf extracts of A.indica is shown to be more effective at combating gram-positive bacteria including streptococcus faecalis[9], on the other hand our study shows better antibacterial efficacy of this herb but not comparable to TAP. According to Zahir Hussain et al., (2010), A. indica's ethanolic extract showcased highest antimicrobial activity (15mm) against Streptococcus faecalis (later recognized as E. faecalis), which is consistent with the results of our investigation.[19]

Sudhakar et al (2006) investigated the antimicrobial efficacy of E.hirta against streptococcus faecalis with 16mm ZOI whereas in our study the ZOI was 21mm.[20] Prabhu Subramani et al in 2022, concluded that E.hirta exhibits strong antibacterial action against general oral pathogens that is in consonance with our study.[21]

Vasudeva et al. (2017) compared the CH paste to CHX gel for evaluating the disinfection of dentinal tubules against E.faecalis however in our study, CHX gel was compared with different pastes.[22] According to Lakhani et al. (2017) & Ghabraei et al. (2018), CHX is more effective than CH[23] and according to Hemanshi kumar et al. (2013), 1% CHX gel is effective than CH against E.faecalis whereas our study was contrary to these studies.[11]

In the root canal therapies, systemic drug administration is least effective than local administration of antibiotic drugs.[24]

Govindaraju et al. (2021) reported that TAP demonstrated a greater ZOI than CH against E. faecalis. Conversely TAP's antibacterial effectiveness was solely evaluated against E. faecalis in our investigation, and the results are comparable to those of that study.[5]

In this study, TAP showed more antimicrobial efficacy followed by E.hirta, CH, A.indica and CHX. The result is in accordance with Mohammad Ali Mozayeni et al. (2020) as he claimed that TAP showed better antibacterial efficacy than CHX gel & CH against E.faecalis.[4]

6. Conclusion

In line with the study's outcomes, among the intracanal medicaments compared, TAP has shown the highest efficacy against E.faecalis and E. hirta also possess better efficacy than the other medicaments. Hence E.hirta can be utilized as a substitute intracanal medicament.

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