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Comparative Evaluation of Antimicrobial Effectiveness of Various Obturating Materials in Primary Teeth - An In-Vitro Study.

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Abstract

Background: The primary goal of endodontic treatment in primary teeth with non-vital pulp is to eliminate infection and to retain the tooth in a functional state until their normal exfoliation time without endangering the permanent dentition. The complexity of root canal system in primary teeth presents a discerning problem for chemo-mechanical preparation. Thus, for optimal success of endo dontic treatment in infected primary teeth, obturating materials having a potent bactericidal effect and the capacity to resorb along with the roots of primary teeth are advocated.

Aim: The aim of this study is to evaluate and compare the antimicrobial activity of various obturating materials.

Materials and Methods: Anti-microbial efficacy of Endo flas, Met apex, Zinc oxide with Aloe vera, Zinc oxide with Propolis, Triple antibiotic paste and Zinc oxide Eugenol was evaluated against Enter ococcus

faecalis, Escherichia coli, Staphylococcus aureus, Pseud omonas aeruginosa by Agar diffusion method. These materials were tested at 24 hours after manipulation and results were reported as diameter of growth inhibition zone of each obturating material.

Statistical Analysis: Statistical analysis was carried out by one-way ANOVA using software SPSS (Statistical Package for Social Sciences) version 16 with Tukey's post-hoc test to compare the statistical difference between anti-microbial effects of materials tested. A p-value of less than 0.05 was considered statistically significant.

Results: Triple Antibiotic Paste (3Mix) was found to have superior anti-microbial activity against all the four micro-organisms followed by Endo flas, Zinc oxide with Propolis, Zinc oxide Eugenol, Met apex and Zinc oxide with Aloe Vera.

Conclusion: Triple Antibiotic paste can be used in necrotic primary teeth with root resorption, severe bone loss or where the prognosis of conventional pulpectomy procedure is considered to be poor.

Keywords: Anti-microbial, obturating materials, pulp ectomy, Triple Antibiotic paste.

Introduction

The primary teeth with pulpal and periapical issues, should be retained until they naturally exfoliate.[1] When the radicular pulp exhibits clinical signs of hyper emia after coronal pulpal amputation or when there is evidence of radicular pulpal necrosis, with or without caries involvement, pulpectomy is indicated for primary teeth with carious pulp exposures. [2]

Micro-organisms and their by-products are considered the major cause of pulpal and peri radicular patho logies. Ade quate root canal debride Ment, anti - microbial irrigants, anti-bacterial filling materials,[1] and intra canal Medi caments to maintain cleanliness between treat ments are among the methods for reducing or elimi nating the patho genic bacteria.[3] Various root canal filling materials for primary teeth have been tested; the most commonly utilised and easily accessible materials are zinc oxide eugenol, calcium hydroxide, iodoform-based pastes, and various combinations of these mate rials.[4]

Therefore, the current study was an attempt to evaluate and compare different root canal filling materials viz., Endoflas, Met apex, Zinc oxide [ZO] + Propolis, Zinc oxide + Aloe Vera gel, Zinc oxide eugenol [ZOE], Triple anti-biotic paste for their anti-microbial efficacy against four micro-organisms - Escherichia coli [E. coli], Pseudo monas aeruginosa [P. aeruginosa], Staphylo coccus aureus [S. aureus] and Enter ococcus faecalis [E. faecalis] - that are frequently isolated from infected root canals of primary teeth.

Materials and Methods

This in-vitro study was carried out in Department of Pedodontics and Preventive Dentistry in collaboration with Department of Microbiology. A sample size of 30 for each test organism, with 5 samples of each material being tested against each bacterial isolate was selected for the study. The test materials were either obtained in a premixed form (Endoflas, Met apex) or mixed using stan dardized powder-liquid ratios (ZOE, ZO + Propolis + Distilled water, ZO + Aloe vera gel, 3Mix [Metro nidazole, Cipro floxacin and Doxycycline] + Propylene Glycol).

The standard bacterial strains were obtained from Kehloor Bio sciences and Research Centre (KBRC), Ghumarwin, Bilaspur, Himachal Pradesh, India and are listed below with their reference numbers. These strains were further divided into four groups. Group A: *E. coli* (KBRC003), Group B: P. aeruginosa (KBR C002),

Group C: S. aureus (KBR C001), Group D: E. faecalis (KBR C005).

Agar diffusion assay

The microbiological assays were performed under aseptic conditions inside a sterilized Laminar Air Flow Chamber. Bacterial inoculums were prepared by sus pending 3-4 loops of each organism from freshly prepared slants and transferring into respective test tubes containing 5ml of normal saline and mixed well. The turbidity of each suspension tube was adjusted until it was equivalent to 0.5 McFarland turbidity standard corresponding to approximately 1.5 x 10^8 CFU/ml. Four suspensions of the four microbial strains used in the study were prepared using this method.

The antibacterial efficacy of obturating material in each group against the four bacterial strains was compared using Agar Diffusion Test.

For Group A (E. coli)

Muller-Hinton agar plates were pre-dried in an incubator for 30 minutes and inoculated by lawn culture. A sterile cotton swab was dipped into the microbial suspension, and then streaked two or more times over the entire surface of MHA plate rotating the plate approximately 60^0 each time to an ensure even distribution of inoculum. Culture plates were allowed to dry for 30 minutes. Six equidistant wells, 6 mm in diameter and 4 mm in depth were made in agar plates using a sterile agar puncher. A sterile spatula and glass slab was used for mixing the ob turating materials in creamy consistency. The evaluation of antimicrobial activity of six testing materials (1, 2, 3, 4, 5, and 6) was performed using agar well diffusion technique. The test and control materials were placed into 6 wells of each agar plate and were kept at room temperature for 15 minutes for pre-diffusion to take place, and later the plates were incubated at 37°C for 24-48 hours. After incubation, a lack in bacterial coloni zation was observed for each obturating material. It was indicated by clear circular halos surrounding the wells. The diameter of the zones of inhibition in mm around the filling materials was measured after 24 hours of incubation. This procedure was performed five times for each isolate and zones were measured independently by one observer.

Similarly for Group B (P. aeruginosa), Group C (S. aureus), Group D (E. faecalis), the experiment was repeated five times for more accuracy and effectiveness. Mean zone of inhibition for each material-microbial strain combination was then calculated.

Results

Statistical analysis was carried out by one-way ANOVA using software SPSS (Statistical Package for Social Sciences) version 16 with Tukey's post-hoc test to compare the statistical difference between antimicrobial effects of materials tested. A p-value of less than 0.05 was considered statistically significant.

The zones of inhibition produced by the test materials against the selected microorganisms are presented in Table 1.

Table 1: Summary of antimicrobial assay of six obturating materials (1, 2, 3, 4, 5 and 6) against four different test isolates.

Test Materials	Diameter of zones of inhibition (mm) against test isolates																			
	E. coli.					P. aeruginosa					S. aureus					E. faecalis				
1	23	22	24	23	24	22	21	22	22	23	30	29	28	29	28	28	29	29	28	29
2	1	12	13	13	12	11	10	12	11	10	12	9	10	9	10	12	10	11	11	11

	2																			
3	21	20	21	19	20	17	17	16	17	17	21	21	22	21	21	17	18	18	17	18
4	11	12	12	11	11	9	11	11	10	10	11	8	9	8	10	12	13	13	12	13
5	13	11	12	13	13	8	7	9	8	7	12	13	14	13	14	15	14	15	15	15
6	55	54	54	55	55	58	58	57	57	58	58	57	56	57	56	56	55	55	54	55

1. Endoflas 2. Met apex 3. Zinc oxide powder + Propolis+ Distilled water 4. Zinc Oxide powder + Aloe vera gel 5. Zinc Oxide Eugenol 6. 3Mix + Propylene Glycol

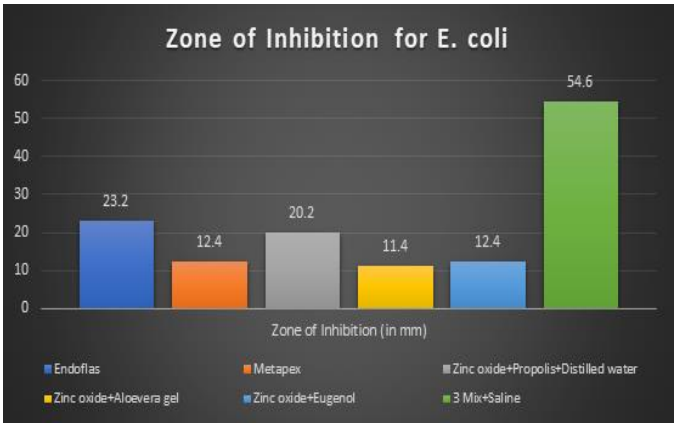
2. The average zone of inhibition against E. coli (Group A) that each test material produced is shown in GRAPH 1. The maximum zone of inhibition was seen with 3Mix + Propylene Glycol (54.60 ± 0.54 mm) whereas the least zone of inhibition was seen with Zinc Oxide+Aloe Vera Gel (11.40 ± 0.54 mm). There was a statistically signifi cant variation in the zone of inhibition between the various study groups on intergroup comparison ($p < 0.001$).

The mean zone of inhibition produced against P. aeruginosa (Group B) is shown in GRAPH 2. The maximum zone of inhibition was seen with 3Mix + Propylene Glycol (57.60 ± 0.54 mm) whereas the least zone of inhibition was seen with Zinc Oxide+Eugenol (7.80 ± 0.83 mm).

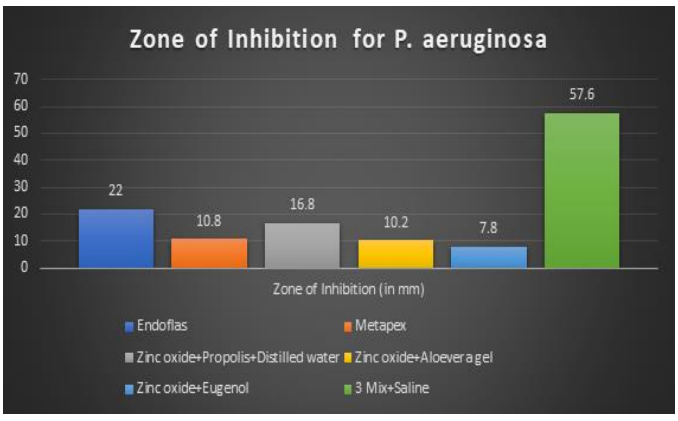
The statistical analysis with ANOVA showed that there was a statistically significant difference in the zone of inhibition among the study groups ($p<0.001$).

The mean zone of inhibition against S. aureus (Group C) is shown in GRAPH 3. Maximum zone was seen with 3Mix+ Propylene Glycol (56.80±0.83 mm) whereas the least zone of inhibition was seen with Zinc Oxide + Aloe vera Gel (9.20 ± 1.30 mm). A statistically significant difference in the zone of inhibition among the study groups ($p<0.001$) was observed.

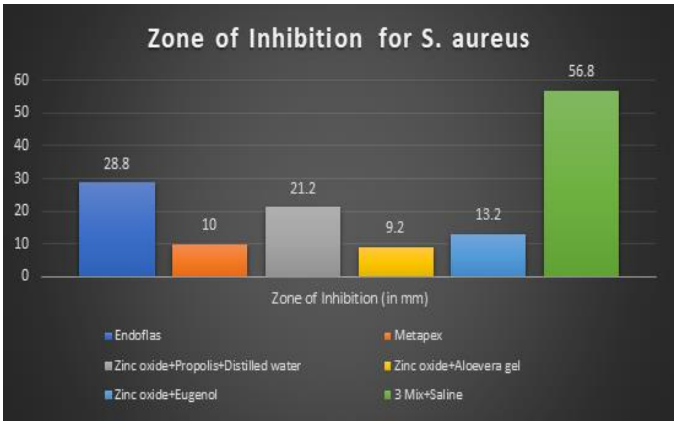
The average zone of inhibition produced against E. faecalis (Group D) by each material is shown in GRAPH 4. 3Mix+Propylene Glycol (55.0±0.70 mm) produced the maximum zone whereas the least zone of inhibition was seen with Met apex (11.0 ± 0.70 mm) and a statistic ally significant difference was observed among the study groups ($p<0.001$).



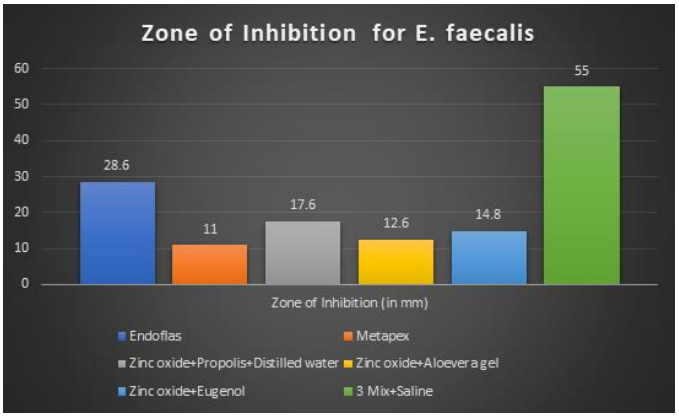
Graph 1: Zone of inhibition (in mm) for E. coli



Graph 2: Zone of Inhibition for P. aeruginosa.



Graph 3: Zone of inhibition for S. aureus.



Graph 4: Zone of inhibition for E. faecalis.

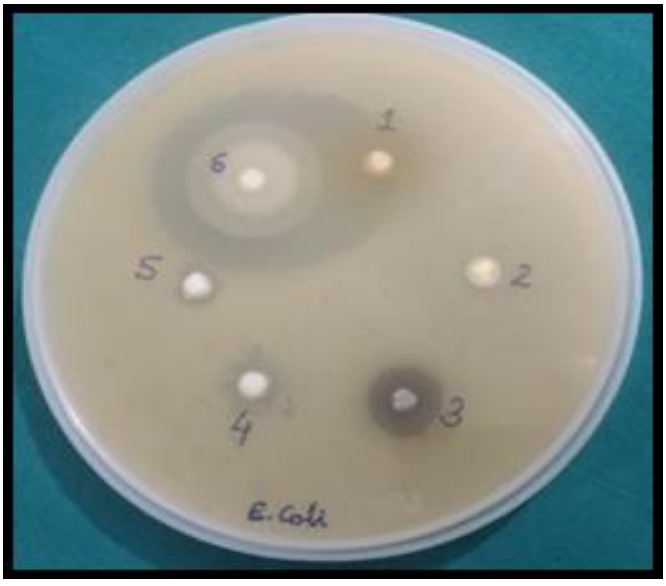


Figure 1: Zones of in hibition representing the anti-bacterial activity of test materials (1,2,3,4,5,6) against E. coli.

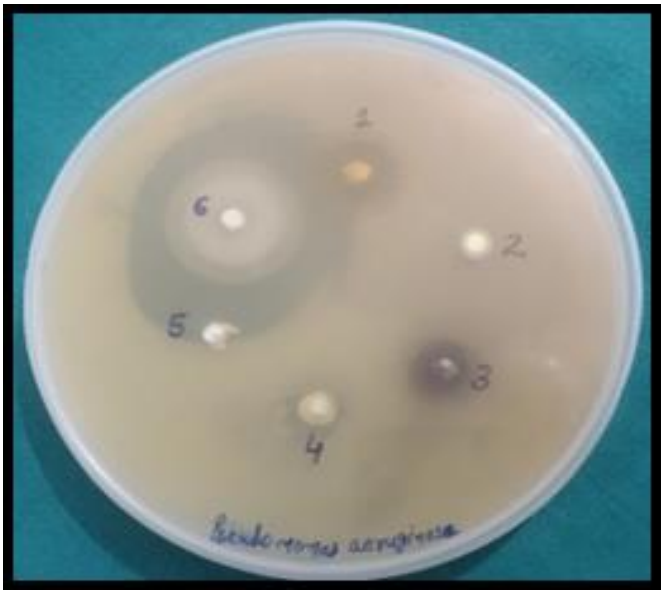


Figure 2: Zones of inhibition representing the anti-bacterial activity of test materials (1,2,3,4,5,6) against P. aeruginosa.

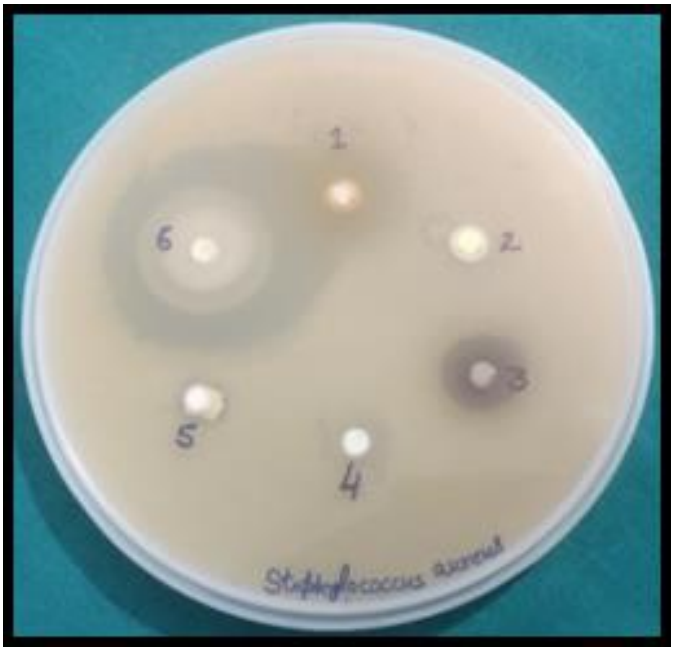


Figure 3: Zones of inhibition representing the anti-bacterial activity of test materials (1,2,3,4,5,6) against S. aureus.

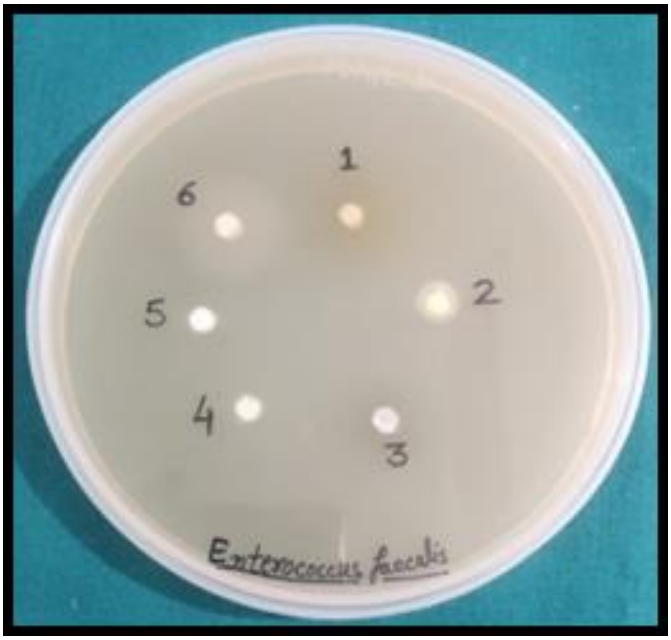


Figure 4: Zones of inhibition representing the anti-bacterial activity of test materials (1,2,3,4,5,6) against E. faecalis.

Discussion

The success or outcome of root canal treatment in permanent teeth is determined by appropriate bio mechanical preparation, whereas the success of pulp ectomy in a deciduous tooth is determined by the resorb able nature and anti-microbial properties of the filling material, which can neutralize any remaining pulpal tissue and micro-organisms.[5]

For decades, there has been debate about the best root canal filling material for primary teeth.[6] Numerous materials have been proposed, but no single material has met all requirements.[7] The present study evaluated the anti-microbial potential of some materials commonly used for filling root canals of primary teeth, such as, Zinc oxide eugenol paste, Met apex, Endoflas and a few newer materials that have been successfully used in primary tooth obturation like Triple antibiotic paste, Zinc oxide+ Propolis and Zinc oxide+ Aloe vera gel.

Various authors reported the presence of facultative anaerobic bacteria and strictly anaerobic bacteria (both gram positive and gram negative) invading the dentinal mass and accessory canals, reaching the periodontium, and forming bacterial biofilm at the apical region. Some times bacterial agglomerations detach from the bacterial biofilm, resulting in planktonic bacteria that can cause distant infections.[8] The genera that most frequently persist in infected root canals include enter ococci, staphylococci and Gram-negative enteric rods.[9] Therefore, four micro-organisms which are commonly isolated from infected root canals of primary teeth - P aeruginosa, E coli, E faecalis and S aureus were selected for the current study. E faecalis can be found in the oral cavity of children through contamination, such as from a pacifier, and is part of the micro biota of infected canals of primary teeth. Micro-organisms such as E coli, E faecalis, and S aureus serve as a reference in quality control methods for antimicrobial sensitivity tests.[6]

The findings of this investigation are addressed in relation to the antibacterial effectiveness of several obturating materials against four bacterial isolates. The strongest antimicrobial effect against E coli (Figure 1) was exhibited by 3 Mix with Propylene Glycol followed by Endoflas, Zinc oxide with Propolis, Met apex, Zinc oxide eugenol and Zinc oxide with Aloe vera. Results of the present study were similar to another in vitro study conducted by Fidalgo [10] and Takushige [10] which also observed good clinical outcome of 95% with 3 Mix. The study's good clinical result could be attributed to the fact that 3Mix can readily dissipate through accessory canals, porosities, and permeability in the pulpal floor region and produce a sterile zone, which is expected to stimulate tissue repair.[11] The zone of inhibition produced by Endoflas was higher than that of all the other test materials except for 3Mix. These results were in accordance with the study conducted by Ramar and Mungara [12] and Sapna H [6], which stated that Endoflas

moderately inhibited the gram-positive and gram-negative organisms. Endoflas' signifi cant antimicrobial activity was likely due to the presence of iodoform and eugenol, both of which have antibacterial action. Eugenol acts by causing protein denaturation, whereas iodoform functions as an oxidising agent.[13] Zinc oxide + Propolis also showed good antimicrobial activity against E coli. Similar results were obtained in a study conducted by Roja Ramya KS [14] wherein Zinc oxide - propolis mixture has demonstrated a success rate of 95% and 93.8% respectively. This success might be credited to the fact because Flavonoids, caffeic acid, benzoic acid, and cinnamic acid in propolis probably act on the microbial cell wall, causing functional and structural damage.

In terms of the mean zone of inhibition against P aeruginosa (Figure 2), 3 Mix with Propylene Glycol had the highest value, followed by Endoflas, Zinc oxide + Propolis, Met apex, Zinc oxide + Aloe vera, and Zinc oxide eugenol, which had the lowest value. The results of this study were in correspondence with the results obtained by Prabhakar AR [15] and Nanda R,[10] in treating infected primary molars following non-instrumentation endo dontics employing a combination of antibiotics. Reason being the complex bacterial composition of the infected root canals according to a study by Hoshino et al. [16] Since the majority of bacteria in the deep layers of infected dentin of the root canal wall consist of obligate anaerobes, metronidazole was selected as the first choice among the antibacterial drugs. Metro nidazole even at high con centrations cannot kill all the bacteria indicating the necessity of other drugs. Thus, Ciprofloxacin and Doxycy cline, in addition to Metronidazole, were added to sterilize infected root dentin.[15] The zone of inhibition produced by Endoflas was higher than that of all the other test materials except for 3Mix. Similar results have been reported by Goel H [5] and Rewal N. [18] This could be attributed to the fact that even after the material sets, surface hydrolysis of the chelate (zinc eugenol ate) results in release of eugenol, thus explaining the effective antibacterial activity of this substance even after 72 hours.[13] Zinc oxide with Propolis showed greater zone of inhibition than Met apex, Zinc Oxide+Aloe vera and Zinc oxide Eugenol. Pinocembrin and apigenin are flavanoids found in propolis. Isolated pin ocembr in has been shown in numerous studies to have anti-microbial effect against S. aureus, E. faecalis, and Pseudo monas aeruginosa. Iso lated apigenin inhibits gram-negative bacteria notably P. aerug inosa and Klebsiella pneumoniae.[18] Zinc oxide eugenol produced the least zone of inhibition. This is partly in accordance with a study by Cox Jr ST. [19] which showed that gram - positive micro - organisms were sensitive to ZOE but not the gram-negative micro-organisms.

The strongest antibacterial effect, or the largest zone of inhibition against Staphyl ococcus aureus (Figure 3) was demo nstrated by 3 Mix with Propylene Glycol, which was followed by Endoflas, Zinc oxide with Propolis, Zinc oxide eugenol, Met apex, and Zinc oxide with Aloe vera. This result coincides with Barja-Fidalgo F [20] and Sato I [21] who reported that 3Mix showed the largest inhibition zones against E. faecalis, E. coli, S. aureus and S. mutans. The extensive use of 3Mix locally may account for its potent antibacterial properties.[12] A study conducted by Grange and Davey [22] showed the antimicrobial efficacy of Propolis against Enter ococcus species and Staphylo coccus aureus. A study conducted by Wasnik MB [23] and Cox Jr ST [19] showed strong inhibitory effect of Zinc oxide Eugenol against S. aureus (gram-positive bacteria).

Pabla T [24] in his study reported least antimicrobial activity of Met apex. This may be explained by the fact that calcium hydroxide an ingredient of Met apex has been demonstrated to interfere with the antiseptic capacity of dyadic combinations of endodontic Medi caments.[25] However, studies conducted by Mortazavi and Mesbahi, [26] Nurko C and Garcia-Godoy F,[27] have reported high clinical success rates with Met apex.

The least amount of *S*. aureus suppression was seen with Zinc Oxide+Aloe vera. This ran counter to research done by Parthasarthy G. [28] Antrokinon, one of the active components of aloe vera, is considered to have antiviral and anti-bacterial properties with minimal cytotoxicity. [29]

E. faecalis is found in 4 to 40% of initial endo dontic infections, and failed root canal treatment cases are nine times more likely to have E. faecalis than primary endo dontic infections.[30] Figure 4 illustrates that 3Mix produced the largest zone of inhibition against E. faecalis, followed by Endoflas, Zinc oxide and Propolis, Zinc oxide eugenol, Zinc oxide and aloe vera, and Met apex. The efficiency of antibiotic drugs in eradicating E. faecalis bio films is demonstrated in a study conducted by Lima KC. [31] This result of this study was consistent with a study by Pandranki J,[32] which showed that Endo flas is very effective against a resistant endo dontic pathogen, E. faecalis. Study conducted by Fuks A [33] also reported high success rate of Endoflas. Zinc oxide+ Propolis showed greater inhibitory activity than Zinc oxide eugenol. This was contrary to research conducted by Rahman EF [34] which showed the inhibitory zone diameter of zinc oxide eugenol to be greater than that of zinc oxide propolis against Enterococcus faecalis. Greater zone of inhibition was produced by ZOE than by ZO+ Aloe vera and Met apex. In another study conducted by Sapna H [6] and Bonow ML,[35] ZOE showed medium inhibition of S mutans, E coli and E faecalis. According to a study conducted by Kriplani R et al.,[1] Zinc oxide+ Aloe vera showed medium inhibitory effect against both Gram-positive and Gram-negative microorganisms. Met apex showed the least inhibitory effect against E. faecalis. These results were in accordance with the studies conducted by Tchaou WS [36] and Pabla T. [24] A reduced in-vitro anti-micro bial activity of calcium hydroxide paste against poly micro bial cultures using agar diffusion assay has been reported by Rezende GP [37]. Although the high pH of calcium hydroxide should inhibit the growth of bacteria, there is a possibility that this pH will be neutralized by blood or culture media buffers in in-vitro experiments.

Conclusion

In conclusion, 3Mix was found to have superior anti-microbial activity against all the four micro-organisms followed by Endoflas, Zinc oxide with Propolis, Zinc oxide Eugenol, Met apex and Zinc oxide with Aloe Vera. 3Mix can be preferred over conventional endo dontic treatment in necrotic primary teeth with resorbed roots, significant mobility, or where furcal bone loss extends to the succedaneous permanent tooth. Endoflas and Zinc oxide+ Propolis can be used as an alternative to Zinc oxide Eugenol. Endodontic infections are complex in terms of the microflora and their interactions. The effect of test filling materials against a single strain may not be effective against a mixed variety of infection. The use of artificial media also plays an important role in determining the experi mental results. Even Agar diffusion Assay has got its own limitations. It is possible that different results might have obtained if other methods of testing antimicrobial activity i.e., Agar dilution method, Direct contact test etc. were employed.

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