

Original Research Article

Evaluation of antimicrobial efficacy of an herbal preparation vs triple antibiotic paste against *E. faecalis*: an *in vitro* study

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ABSTRACT

Background: *Enterococcus faecalis* is a major pathogen responsible for persistent endodontic infections and root canal treatment failure due to its resistance and ability to survive harsh intracanal conditions. Triple antibiotic paste (TAP) is widely used for canal disinfection, but concerns related to antibiotic resistance, tooth discoloration, and cytotoxicity have led to interest in herbal alternatives. This study evaluated the antimicrobial efficacy of a herbal formulation containing Turmeric, Arjuna bark and Clove oil, against *E. faecalis* and compared it with TAP.

Methods: An *in vitro* well diffusion assay was performed using *E. faecalis* (ATCC 29212) inoculated on Mueller–Hinton agar. Three concentrations (20 mg/ml, 50 mg/ml, and 100 mg/ml) of the herbal extract were tested, and zones of inhibition (ZOI) were measured after incubation at 37°C for 48 hours. Minimum inhibitory concentration (MIC) was determined using a microbroth dilution method. TAP served as the positive control.

Results: The herbal preparation demonstrated measurable antimicrobial activity, showing a ZOI of 8 mm (20 mg/ml), 10 mm (50 mg/ml), and 15 mm (100 mg/ml). The MIC value for the herbal formulation was 7.81 µg/ml. TAP showed higher inhibition zones at all tested concentrations.

Conclusions: The herbal formulation exhibited significant antibacterial activity against *E. faecalis*, indicating its potential as a biocompatible intracanal medicament. Although TAP remained more effective at lower concentrations, the herbal preparation offers advantages such as reduced risk of antibiotic resistance and tooth discoloration. Further cytotoxicity testing and clinical studies are required to validate its clinical applicability.

Keywords: Herbal preparation, Triple antibiotic paste, *Enterococcus faecalis*, Antibacterial activity, Pediatric dentistry

INTRODUCTION

Root canal treatment is a common dental procedure aimed at eliminating infection from the root canal system and preserving the natural tooth. The process involves removal of the infected or necrotic pulp, followed by thorough cleaning, disinfection, and sealing of the canal to prevent reinfection.¹ Successful endodontic therapy depends on the complete eradication of microorganisms and the prevention of bacterial recolonization within the treated canal. Infection within the root canal typically develops after pulp necrosis resulting from dental caries, trauma, periodontal involvement, or previous endodontic interventions.² Once established, bacteria and their by-

products can migrate through the apical or lateral foramina and induce periradicular inflammation.^[3] Among the various microbial species identified in endodontic infections, *Enterococcus faecalis* is frequently associated with persistent and recurrent infections, often leading to treatment failure.^{3,4} This bacterium exhibits remarkable resistance to unfavourable environmental conditions and commonly survives conventional chemomechanical preparation and intracanal medicaments.

TAP, a combination of ciprofloxacin, metronidazole, and minocycline, has been widely used for disinfecting infected root canals. However, its clinical use presents

several drawbacks, including tooth discoloration (attributed to minocycline), potential allergic reactions, and the growing concern of antibiotic resistance.^{5,6} These limitations have prompted the exploration of natural alternatives with comparable antimicrobial efficacy and fewer side effects. Herbal agents have gained increasing attention in endodontics due to their biocompatibility, availability, minimal toxicity, and lower likelihood of inducing microbial resistance. In this study, a novel herbal formulation containing Turmeric (*Curcuma longa*), Arjuna bark (*Terminalia arjuna*), and Clove oil (*Syzygium aromaticum*) was tested for its antimicrobial efficacy against *E. faecalis*. Herbal preparation (TAC) was also compared with the conventional triple antibiotic paste. Thus, this investigation aims to assess whether herbal medicaments can serve as effective and safer alternatives to conventional antibiotic-based intracanal medicaments in endodontic therapy.

METHODS

Study design

This *in vitro* experimental study was conducted to evaluate and compare the antibacterial efficacy of a herbal preparation TAC: Turmeric (*Curcuma longa*), Arjuna bark (*Terminalia arjuna*), and Clove oil (*Syzygium aromaticum*) with Triple Antibiotic Paste (TAP) against *Enterococcus faecalis*.

Ethical approval

The study protocol was reviewed and approved by the Scientific Review Board of Seema Dental College & Hospital, Rishikesh (EC/NEW/ INST/2025/UA/0615). As this was an *in vitro* laboratory-based study, no human or animal subjects were directly involved.

Microorganism and culture conditions

A standard strain of *E. faecalis* (ATCC 29212) was procured from a certified diagnostic supplier and stored at 2–4°C as per manufacturer's instructions. The organism was revived within 48–72 hours and cultured in Mueller-Hinton Broth (MHB). For antimicrobial testing, Mueller-Hinton agar plates were prepared under sterile conditions.

Preparation of herbal extract TAC (Turmeric, Arjuna bark, Clove oil)

Turmeric extract was prepared by collecting organically grown rhizomes from a known farm, washing them thoroughly with distilled water, shade-drying them for 10-14 days, and then grinding them into a fine powder (Figure 1a). Arjuna bark extract was prepared by obtaining disease-free bark from a licensed Ayurvedic shop, shade-drying it for 10–15 days, and then grinding it into a fine powder (Figure 1b). Clove oil was obtained in its pure form from a licensed Ayurvedic shop and used without further processing (Figure 1c). Turmeric, Arjuna

bark, Clove oil all were mixed together in acetone solvent in ratio of 1:10. The mixture was stirred and left to stand for 48-72 hours at room temperature (Cold percolation). The mixture was then filtered through Whatman filter paper, and the solvent was evaporated under reduced pressure. The combined plant extract was dissolved in 10 % DMSO to prepare stock solutions of 20 mg/ml, 50 mg/ml and 100 mg/ml (Figure 2).

Control preparation triple antibiotic paste

It was prepared by mixing Ciprofloxacin, Minocycline, Metronidazole in 1:1:1 ratio after pulverising the tablets in motor pestle and mixing it with distilled water to make the paste.

Agar well diffusion assay

The antibacterial activity was assessed using the agar well diffusion method. Mueller-Hinton agar plates were inoculated with *E. faecalis* using a sterile cotton swab to obtain a uniform lawn culture. Wells of 6 mm diameter were prepared using a sterile cork borer, and 50 µl of the test solutions at different concentrations were dispensed into the respective wells. Plates were incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters. All experiments were performed in triplicate.

Minimum inhibitory concentration assay

The MIC was determined using the broth microdilution method. Two-fold serial dilutions of the extracts (1000–1.95 µg/ml) were prepared in Mueller-Hinton broth in 96-well microtiter plates. Each well received 100 µl of extract and 100 µl of bacterial suspension adjusted to 10⁵ CFU/ml. After incubation at 37°C for 24 hours, microbial growth was assessed visually and confirmed using resazurin dye. The MIC was defined as the lowest concentration showing no visible color change.

Statistical analysis

All data were analyzed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and are presented as mean±standard deviation (SD). One-way analysis of variance (ANOVA) was applied to assess differences in the zone of inhibition of the herbal preparation (TAC) across concentrations. Intergroup comparisons between TAC and TAP for zone of inhibition and MIC were performed using the independent Student's t-test. Statistical significance was set at p<0.05, and p<0.001 was considered highly significant.

RESULTS

From the observations obtained in the present study, TAP demonstrated the maximum antimicrobial activity against *Enterococcus faecalis*, showing the highest mean zone of inhibition of 25.00 mm at 100 mg/ml concentration.

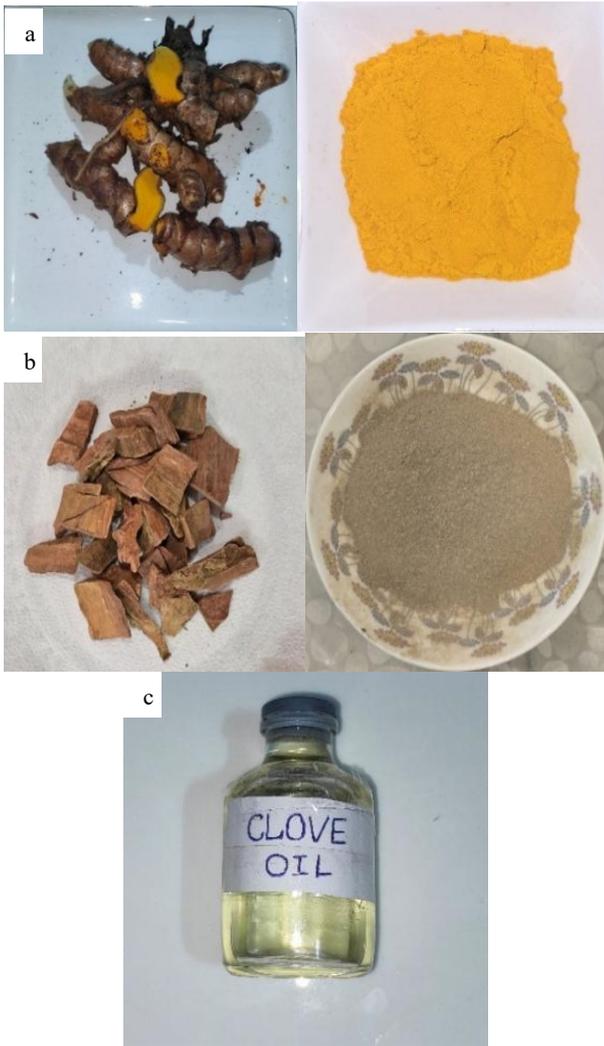


Figure 1 (a-c): Turmeric rhizome and its powder, Arjuna bark and its powder, Clove oil.

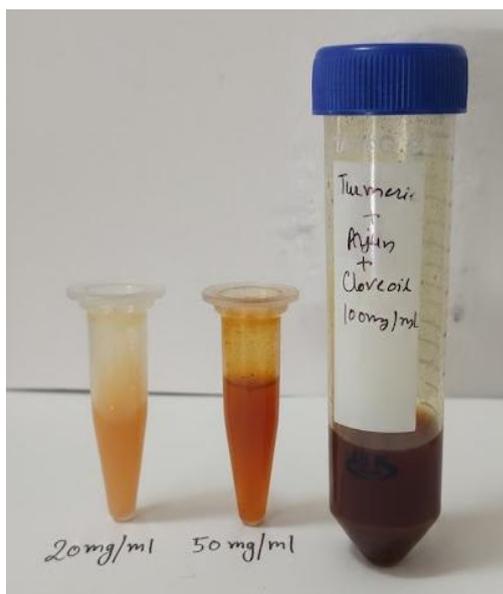


Figure 2: Serial concentration of herbal preparation at 20 mg/ml, 50 mg/ml and 100 mg/ml.

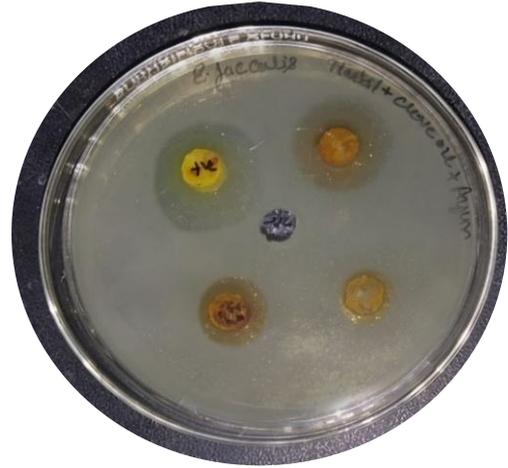


Figure 3: Zones of inhibition produced by TAC and TAP formulations against *E. faecalis*.

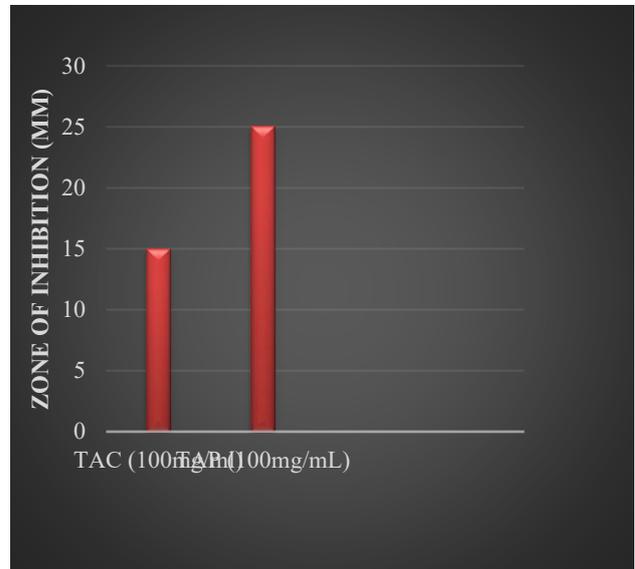


Figure 4: Comparison of zone of inhibition between TAC & TAP.

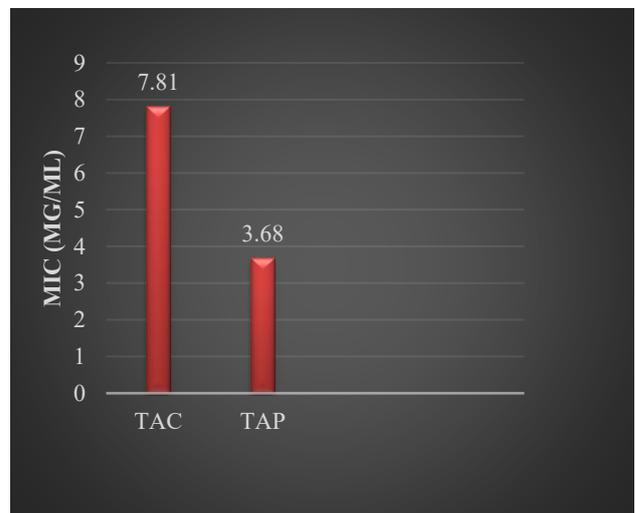


Figure 5: Comparison of MIC between TAC & TAP.

Among the three concentrations of the herbal preparation (TAC: Turmeric, Arjuna bark, Clove oil), the 100 mg/ml concentration showed the greatest antibacterial activity, producing a mean zone of inhibition of 15.24 mm, followed by 10.12 mm at 50 mg/ml and 8.00 mm at 20 mg/ml (Figure 3, Table 1). Although TAP exhibited superior antimicrobial efficacy compared with the herbal preparation, the herbal formulation demonstrated a clear concentration-dependent increase in inhibition against *E. faecalis* (Table 2, Figure 4). The MIC assay further

revealed that the herbal preparation exhibited a MIC of 7.81 µg/ml, indicating measurable antibacterial activity at low concentrations. TAP showed a lower MIC value, confirming its higher potency (Table 3, Figure 5). Overall, TAP produced the greatest inhibitory effect, while the herbal formulation demonstrated significant but comparatively lower antimicrobial efficacy, suggesting its potential as a natural intracanal medicament with advantages such as reduced risk of antibiotic resistance and absence of discoloration.

Table 1: Zone of inhibition of herbal preparation (TAC) at different concentrations.

TAC concentration (mg/ml)	Mean ZOI (mm)	Statistical comparison	P value
20	8.00±SD	One-way ANOVA	<0.05* significant difference
50	10.12±SD		
100	15.24±SD		

One-way analysis of variance (ANOVA); * indicates significant difference at p≤0.05.

Table 2: Comparison of zone of inhibition between TAC & TAP.

Group	Concentration (mg/ml)	Mean ZOI (mm)	Statistical comparison	P value
TAC	100	15.24±SD	Independent t-test	p<0.00* highly significant difference
TAP	100	25.00±SD		

Independent Student's t-test; * indicates highly significant difference at p<0.001.

Table 3: Comparison of MIC between TAC & TAP.

Intracanal medicament	MIC (µg/ml)	Statistical comparison	P value
TAC	7.81	Independent t-test	p<0.001* highly significant difference
TAP	3.68		

Independent Student's t-test; * indicates highly significant difference at p<0.001

DISCUSSION

Enterococcus faecalis is one of the most frequently isolated pathogens in persistent endodontic infections and remains a primary cause of root canal treatment failure.⁷ Its ability to survive in nutrient-deprived environments, penetrate dentinal tubules, and resist conventional chemomechanical preparation makes its elimination difficult.^{3,8,9} Complete eradication through instrumentation and irrigation alone is often impossible, particularly in teeth with open apices and physiologic root resorption. Therefore, intracanal medicaments play a crucial role in reducing microbial load and preventing reinfection.²

TAP, introduced to enhance bacterial elimination in the lesion sterilization and tissue repair (LSTR) concept, combines metronidazole, ciprofloxacin, and minocycline and has been widely used due to its broad-spectrum activity. However, minocycline-induced tooth discoloration and the rising threat of antibiotic resistance have prompted the search for alternative formulations.^{5,6} To overcome these limitations, a modified TAP (MTAP) replacing minocycline with clindamycin has been suggested. Karczewski et al reported that clindamycin exhibits effective antimicrobial properties while

producing significantly less dentin discoloration and reduced cytotoxicity, supporting its suitability as a substitute in TAP formulations.¹⁰ Parallel to the concerns regarding antibiotics, herbal extracts have gained prominence due to their lower toxicity, biocompatibility, longer shelf life, and minimal risk of inducing microbial resistance. Over the past decade, several investigations have supported the antimicrobial potential of plant-derived medicaments against resistant endodontic pathogens, particularly *E. faecalis*. Prabhakar et al demonstrated that turmeric extract possesses substantial antibacterial action without compromising dentin microhardness, making it a potential intracanal medicament.¹¹ Similar findings were supported by subsequent *in vitro* studies evaluating turmeric's efficacy against endodontic microorganisms. Valera et al evaluated herbal extracts against *E. faecalis* and *Candida* species and concluded that although herbal extracts significantly reduced the microbial count, bacteria residing deeper in dentinal tubules were less susceptible. This observation correlates with the present study, where the herbal combination showed measurable antibacterial activity but remained inferior to TAP.¹² Kalaiselvam et al showed that when herbal powders are combined with suitable vehicles (such as methylcellulose), their diffusion and antibacterial efficacy are significantly

enhanced. This reinforces the findings of the current study, where the herbal preparation prepared with 10% DMSO demonstrated concentration-dependent inhibition against *E. faecalis*.¹³ Setty et al explored the antimicrobial activity of nutmeg essential oil and found strong inhibitory effects against endodontic pathogens, indicating that plant-derived volatile oils may exert significant antibacterial action.¹⁴ Although nutmeg was not included in the current formulation, its documented effect supports the growing acceptance of herbal combinations in endodontics. Golla et al reported strong antibacterial activity of herbal combinations against *E. faecalis*, with ginger–nutmeg at 12.5% showing inhibition comparable to MTAP.¹⁵ These results align with the present study, where the turmeric–Arjuna bark–clove oil formulation also demonstrated notable antimicrobial efficacy, further supporting the potential of multi-herbal agents as effective intracanal medicaments.

In the present *in vitro* study, the herbal preparation (TAC: Turmeric, Arjuna bark, Clove oil) demonstrated a mean zone of inhibition of 15.24 mm at 100 mg/ml and showed a MIC of 7.81 µg/ml, indicating appreciable antimicrobial activity. Clove oil, known for its eugenol content, may have contributed significantly due to its proven antibacterial and cell wall-disruptive properties. Arjuna bark, rich in tannins and flavonoids, has also been shown to exhibit antimicrobial activity in several pharmacological studies, Arjuna Bark Study, 2023, validating its use in the current formulation. However, as expected, TAP displayed superior antimicrobial efficacy with the highest inhibition zone of 25.00 mm and lower MIC values. This reaffirms the established potency of TAP and mirrors the findings of earlier studies evaluating its strong activity against *E. faecalis*.

Overall, the findings from the present study, supported by the literature from 2013 to 2024, indicate that herbal preparations possess significant antibacterial activity against *E. faecalis* and may serve as promising adjuncts or alternatives to conventional antibiotic-based intracanal medicaments. While TAP demonstrated superior antimicrobial potency in this study, herbal agents such as the turmeric–Arjuna bark–clove oil combination show strong potential as safer, biocompatible, and resistance-free alternatives for endodontic disinfection.

Limitations

The limitations of the present study include its *in vitro* nature, which cannot fully replicate the intricacies of *in vivo* conditions such as dentinal penetration, buffering effects of dentin, and host immune responses.

CONCLUSION

Within the limitations of this *in vitro* investigation, the herbal formulation comprising turmeric, Arjuna bark, and clove oil demonstrated measurable antibacterial activity against *E. faecalis*, with a clear concentration-dependent

increase in efficacy and an MIC of 7.81 µg/ml. Although TAP exhibited significantly greater antimicrobial potency at all concentrations, the herbal preparation showed substantial inhibitory effects, indicating its potential as a biocompatible intracanal medicament. Its advantages including absence of tooth discoloration, reduced risk of antibiotic resistance, and natural origin further support its promise as an alternative or adjunctive agent in endodontic disinfection. However, given that this study was conducted under controlled laboratory conditions, further research involving cytotoxicity assessment, dentinal tubule penetration, biofilm models, and clinical trials is essential before recommending its routine clinical use.

Recommendation

Further studies should evaluate the cytotoxicity, dentinal tubule penetration, and biofilm-disrupting ability of the Turmeric, Arjuna bark & Clove oil formulation under conditions simulating the clinical environment. If validated through *in vivo* and clinical studies, this herbal medicament may serve as a safe, non-discoloring, and antibiotic-free intracanal alternative in pediatric endodontics.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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