

Comparative Evaluation Of Antibacterial Activity Of Calcium Hydroxide Combined With Three Different Ayurvedic Oils As Vehicle Against *Fusobacterium Nucleatum* – In Vitro Study

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Abstract

Background: Apical periodontitis is primarily caused by microbial invasion of the root canal system, with *Fusobacterium nucleatum* being one of the key pathogens linked to endodontic treatment failure. Calcium hydroxide [Ca(OH)₂] is a widely used intracanal medicament, but its limited efficacy against certain anaerobes necessitates the exploration of alternative or adjunctive agents. Herbal oils such as Arimedadi, Dashmool, and Liquorice oil have shown promising antimicrobial potential in traditional medicine.

Aim: To evaluate the antibacterial efficacy of calcium hydroxide combined with three different Ayurvedic oils—Arimedadi oil, Dashmool oil, and Liquorice oil—against *Fusobacterium nucleatum* in vitro.

Materials and Methods: An in vitro study was conducted using *F. nucleatum* (MTCC 23726). The antimicrobial activity of calcium hydroxide mixed with each oil was assessed using agar well diffusion and broth microdilution methods. The inhibition zones were measured, and results statistically analyzed using one-way ANOVA and Tukey's post hoc test ($p < 0.05$).

Results: All calcium hydroxide-oil combinations exhibited antibacterial activity against *F. nucleatum*. Glycerine (control) demonstrated the highest inhibition (19.62 ± 0.89 mm), followed by Liquorice oil (15.85 ± 0.91 mm), Dashmool oil (15.35 ± 0.21 mm), and Arimedadi oil (13.60 ± 0.14 mm). Statistically significant differences were observed between groups ($p < 0.01$). Liquorice and Dashmool oils showed comparable efficacy, while Arimedadi oil showed the least antibacterial activity.

Conclusion: The incorporation of Ayurvedic oils, particularly Liquorice and Dashmool oils, enhances the antimicrobial efficacy of calcium hydroxide against *F. nucleatum*.

INTRODUCTION:

Apical periodontitis is a localised inflammatory condition affecting the periradicular tissues, primarily induced by microbial invasion of the root canal system. (1) The complexity and anatomical variability of the root canal often present significant challenges to effective debridement and disinfection during endodontic treatment. Consequently, residual microbial biofilms may persist even after thorough chemomechanical preparation, posing a risk for treatment failure. (2) To address these limitations, intracanal antimicrobial medicaments have been proposed as adjunctive agents to enhance root canal disinfection and improve clinical outcomes.

Calcium hydroxide is commonly utilised in dentistry as a root canal medicament. It plays a key role in eliminating bacteria that cannot be fully removed through instrumentation and irrigation alone. (3) Known for its antibacterial properties and its capacity to promote dentin remineralisation, calcium hydroxide also contributes to the healing process of periapical lesions. (4)

Root canal treatment failures are often linked to the continued presence of microbial infections within the root canal system, with or without involvement of the periradicular region. Remaining bacteria and their toxic byproducts, or coronal leakage, are common causes of these failures. (5) Among the bacteria frequently associated with unsuccessful root canal procedures are *Fusobacterium nucleatum* and *Enterococcus faecalis*. (6)

Fusobacterium nucleatum is a Gram-negative, spindle-shaped rod bacterium that has been implicated in various dental infections, including dental abscesses, periapical inflammation, and failed endodontic treatments. (7) Its ability to cause disease is tied to its virulence factors and its capacity to persist within the root canal environment. One of its notable virulence mechanisms is the production of poly-gamma-glutamate, which helps it survive under harsh conditions. (8) Factors such as oxygen levels, pH, and the presence of nutrients influence its pathogenic behaviour. Despite the use of calcium hydroxide, obligate anaerobes like *Fusobacterium* species often remain dominant, highlighting the limitations of this medicament. (9,10)

Calcium hydroxide ($\text{Ca}(\text{OH})_2$) is a strong alkaline compound with a pH ranging between 12.5 and 12.8 when dissolved in a solvent. It dissociates into calcium ions (Ca^{2+}) and hydroxyl ions (OH^-). In root canal treatment, calcium ions help stimulate dentin formation, while the hydroxyl ions elevate the pH to a highly alkaline level, which disrupts bacterial cell walls, leading to their destruction. (3)

For optimal effectiveness, the medicament should maintain extended contact with the dentin and penetrate deeply into the dentinal tubules. This effectiveness is influenced by the contact angle between the medicament and the dentinal surface, as well as the type of vehicle used to deliver the medicament to the targeted site. (11)

Given the limited antibacterial efficacy of calcium hydroxide, there is a need for further investigation into adjunctive materials that can be combined with $\text{Ca}(\text{OH})_2$ to enhance its antimicrobial activity while preserving its ability to support the regeneration of dental hard tissues.

There is a growing trend toward using Ayurvedic and herbal medications for their antimicrobial properties, offering safe and economical alternatives for treating various diseases. Dashmool oil, along with Arimedadi Taila and liquorice oil, has antibacterial properties beneficial for oral health. (12,13) However, there is a lack of extensive scientific research on Ayurvedic treatments for oral diseases, and only a few studies have investigated the antibacterial effects of herbal extracts used as intracanal medicaments in endodontics. So the aim of this study is to evaluate the antimicrobial efficacy of Aremidadi Oil, Dashmool Oil, and liquorice oil as a vehicle with calcium hydroxide against *Fusobacterium nucleatum*.

MATERIALS AND METHODS

The study was conducted in vitro in an experimental laboratory at Trichy Research Institute of Biotechnology, Tiruchirappalli, Tamil Nadu, using *F.nucleatum-23726(MTCC)* as the study sample.

a. Nutrient Agar Medium

A total of 2.8 grams of commercially available Nutrient Agar (HiMedia) was dissolved in 100 ml of distilled water to prepare the medium. The solution was then sterilised by autoclaving at 121°C under 15 lbs pressure for 15 minutes. After autoclaving, the medium was thoroughly mixed and poured into 100 mm Petri dishes (approximately 25–30 ml per plate) while still in the molten state.

b. Nutrient broth

To prepare nutrient broth, 2.8 grams of commercially available nutrient medium (HiMedia) was added to 100 ml of distilled water and heated until fully dissolved. The prepared medium was then dispensed as required and sterilized by autoclaving at 121°C under 15 lbs pressure for 15 minutes.

c. Calculation of MICs

The broth microdilution assay method was used to determine the MICs for the susceptibility of *Fusobacterium nucleatum* in sterile disposable flat-bottomed 96-well microtiter plates.

The agar diffusion method was employed to assess the antibacterial activity against *Fusobacterium nucleatum*. Petri dishes containing 20 ml of nutrient agar were inoculated with a 24-hour bacterial culture, adjusted to a 0.5 OD value in accordance with the McFarland standard. Wells were created in the agar, and various concentrations of the test samples were introduced. The plates were then incubated at 37°C for 24 hours. Antibacterial efficacy was determined by measuring the diameter of the inhibition zones surrounding the wells.

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test with SPSS version 22 (IBM® Inc., Pvt. Ltd.). A significance level of $P < 0.05$ was considered statistically meaningful.

RESULTS

Following 24 hours of incubation, the presence of clear inhibition zones confirmed that all four solvent-calcium hydroxide mixtures exhibited antibacterial activity against *Fusobacterium nucleatum*. The mean diameters of the inhibition zones (fig 1) for each experimental group are summarized in Table 1.

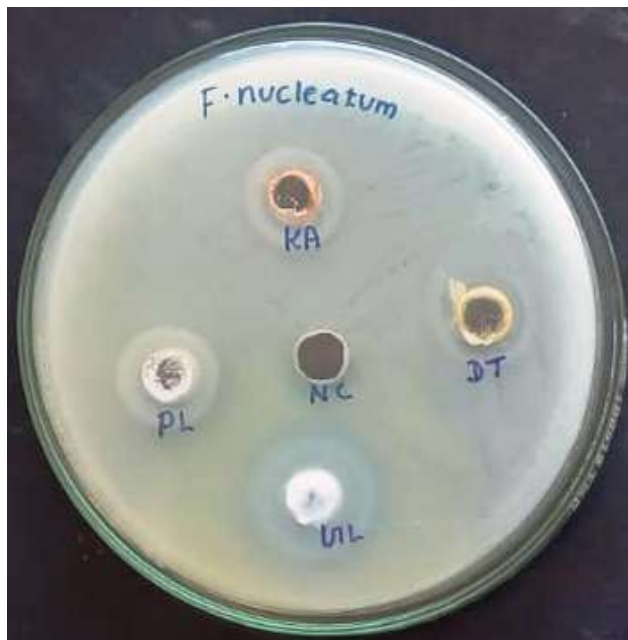


Figure 1 : Effect of sample KA, PL, DT, GL against *F.nucleatum* (KA- Arimedadi oil, PL-Liquorice oil, DT- Dashmool oil, GL-Glycerine)

Among the four groups tested, Glycerine showed the highest mean inhibition (19.62 ± 0.89), followed by Liquorice oil, Dashmool oil, and Arimedadi oil (4.72 ± 2.22). One-way ANOVA showed a significant difference between groups ($F = 76.961$, $p < 0.01$), indicating varied antibacterial effects. (Table 2)

Post hoc analysis (Tukey's HSD) revealed that Arimedadi oil showed significantly lower inhibition compared to Dashmool oil ($p = 0.039$), Glycerine ($p = 0.011$), and Liquorice oil ($p = 0.040$). Glycerine showed significantly higher inhibition than both Dashmool oil ($p < 0.01$) and Liquorice oil ($p = 0.032$). There was no significant difference between Dashmool oil and Liquorice oil ($p = 0.333$). (Table 3)

Table 1. Comparison of zone of Inhibition (mm) of different herbal oils against *Fusobacterium nucleatum* strain, (N=12).

Test sample	n	Mean (SD) of zone of inhibition (mm) F. nucleatum strain	F- statistics	p- value
Arimedadi oil	3	13.60(0.14)	17.37	<0.01
Liquorice oil	3	15.85(0.91)		
Dashmool oil	3	15.35(0.21)		
Glycerine	3	17.0(0.70)		

*p- value less than 0.05 is considered statistically significant.

Table 1 demonstrates the comparative antimicrobial activity of various herbal oil formulations against *Fusobacterium nucleatum*, as measured by the zone of inhibition. Among the test samples, Liquorice oil exhibited the largest zone of inhibition (15.85 ± 0.91 mm), followed by Dashmool oil (15.35 ± 0.21 mm), while Arimedadi oil showed the smallest zone (13.60 ± 0.14 mm), indicating comparatively lower antimicrobial efficacy. Glycerine, used as a negative control, showed the largest zone (17.00 ± 0.70 mm), likely due to non-antimicrobial factors. One-way ANOVA revealed a statistically significant difference among the groups ($F = 17.37$, $p < 0.01$), confirming meaningful variation in antimicrobial effects across formulations.

Table 2: Comparison of Mean inhibition values Among Study Groups, (N=12).

Study group	n	Mean (SD) of % inhibition	F- Statistics	p- value
Arimedadi oil	3	4.72(2.22)	76.961	<0.01
Dashmool oil	3	14.49(0.63)		
Glycerine	3	19.62(0.89)		
Liquorice oil	3	15.43(0.27)		

*p- value less than 0.05 is considered statistically significant.

Table 2 presents the comparison of mean percentage inhibition values among the four study groups—Arimedadi oil, Dashmool oil, Glycerine, and Liquorice oil (N = 12). The mean % inhibition was highest for Glycerine (19.62 ± 0.89), followed by Liquorice oil (15.43 ± 0.27), Dashmool oil (14.49 ± 0.63), and the lowest for Arimedadi oil (4.72 ± 2.22). One-way ANOVA demonstrated a statistically significant difference among the groups (F = 76.961, p < 0.01), suggesting the presence of significant variation in inhibitory effects between treatments.

Table 3: Post Hoc Pairwise Comparisons Between Groups (Tukey's HSD), (N=12).

Reference group	Comparison group	Mean difference (95% CI)	p- value
Arimedadi oil	Dashmool oil	-9.77(-18.47, -1.06)	0.039
	Glycerine	-14.90(-22.95, -6.84)	0.011
	Liquorice oil	-10.71(-20.28, -1.15)	0.040
Dashmool oil	Glycerine	-5.13(-8.04, -2.22)	<0.01
	Liquorice oil	-0.94(-3.18, 1.29)	0.333
Glycerine	Liquorice oil	4.19(0.76, 7.62)	0.032

*p- value less than 0.05 is considered statistically significant.

DISCUSSION

Calcium hydroxide is an antibacterial medicament used in root canals. Within the root canal system, it dissociates into calcium and hydroxyl ions upon contact with an aqueous medium. This leads to disruption of the bacterial cytoplasmic membrane, denaturation of proteins, and inhibition of DNA replication. The diffusion of these ions creates an alkaline environment that interferes with bacterial defence mechanisms.(3,14) Calcium hydroxide is reported to have an ability to eliminate microorganisms, such as *E. faecalis*, *F. nucleatum*, *A. naeslundii* and *Candida albicans* by its alkaline stress. Distilled water is the most common solvent for calcium hydroxide. While it has a neutral pH and good solubility, it lacks antibacterial properties and does not enhance the drug's effectiveness.(15) This study is the first to investigate the use of calcium hydroxide in combination with Arimedadi oil, Dashmool oil, and liquorice oil as solvents in endodontic applications. The findings led to the rejection of the null hypothesis, as statistically significant differences were observed among all experimental groups.

Among all tested agents, glycerin—used as the positive control—produced the largest inhibition zone against *Fusobacterium nucleatum*. This finding aligns with the results reported by Siti Rusdiana Puspa Dewi et al.,(16) where the addition of glycerin as a solvent to calcium hydroxide significantly enhanced antibacterial activity. Chemically, glycerin is a trihydric alcohol composed of three carbon atoms and three hydroxyl (OH) groups, which allows it to interact and bind with various substances. Recent studies have highlighted its role in improving the penetration of medicaments into dentinal tubules, thereby enhancing their antimicrobial efficacy.(17) Due to its hygroscopic property, glycerin supports the gradual release of calcium hydroxide ions over an extended duration. Its strong alkalinity also contributes to an elevated pH, which is unfavourable for bacterial survival. For instance, *F. nucleatum* thrives at a pH between 8 and 8.3.(18) Since calcium hydroxide has a pH of around 12.5, the incorporation of glycerin further increases this alkalinity, strengthening its antibacterial potential.

Liquorice has been reported to possess a diverse spectrum of secondary metabolites, such as saponins, flavonoids, isoflavonoids, chalcones, coumarins, aurones, benzofurans, phenols, pterocarpanes, and stilbenes, which are associated with its broad pharmacological potential.(19,20) In endodontics, only a limited number of *in vitro* studies have assessed its role as a root canal irrigant or intracanal medicament. Badr et al. evaluated the antibacterial and cytotoxic properties of liquorice in comparison with calcium hydroxide [Ca(OH)₂]. Their findings revealed that liquorice extract demonstrated a significantly higher inhibitory effect against *E. faecalis* than Ca(OH)₂ when used alone, and its efficacy was further enhanced when combined with Ca(OH)₂.(21)

Mohammad Reza Tabatabaeian et al. reported that liquorice extract exhibited notable antimicrobial activity against *Fusobacterium nucleatum*, with a minimum inhibitory concentration (MIC) of 6.25 mg/ml. The study demonstrated that at concentrations exceeding 25 mg/ml, the extract's antibacterial efficacy significantly outperformed that of 0.2% chlorhexidine ($P < 0.05$), highlighting liquorice as a promising natural candidate for use in oral healthcare and the management of periodontal infections.(22)

Dashmool, meaning "ten roots,"(13) is a classical Ayurvedic formulation composed of roots from both trees and shrubs, including species such as *Patala*, *Gambhari*, *Brihati*, and *Shalparni*. Its therapeutic efficacy has been well-documented in traditional medicine, particularly in the management of ENT (ear, nose, and throat) disorders. The presence of bioactive compounds contributes to its broad pharmacological profile, including anti-inflammatory, antioxidant, antibacterial, analgesic, and sedative properties. These effects make Dashmool valuable in treating various inflammatory and pain-related conditions, such as osteoarthritis, rheumatoid arthritis, and gout. Moreover, its traditional use as an enema highlights its gastrointestinal benefits, particularly in alleviating symptoms like constipation, bloating, flatulence, and anorexia. The findings of the present study further support the antimicrobial potential of Dashmool, suggesting its relevance as a natural adjunct in endodontic applications.(23,24)

Arimedadi oil is commonly utilized in the management of various periodontal conditions and is also recommended for practices like oil pulling or gargling. It has shown therapeutic potential in addressing several oral health issues, including glossitis, aphthous ulcers, dental caries, and gingivitis.(12) The formulation comprises medicinal ingredients such as *Rubia cordifolia* (Manjishtha), *Acacia catechu* (Khadira), sesame oil (*Sesamum indicum*), clove (*Syzygium aromaticum*), among others. Each component contributes distinct therapeutic properties—Khadira is recognised for its astringent and bactericidal effects, with additional roles as an analgesic and anticaries agent. Similarly, Manjishtha exhibits astringent, analgesic, anti-inflammatory, antimicrobial, and blood-purifying actions. The oil is rich in both oil- and water-soluble phytoactive constituents, which are believed to enhance its overall efficacy in oral care. Although arimedadi oil has shown good results in other study by Kumari M et al as an Ayurvedic oil-based root canal sealer against *E faecalis*(25), this study demonstrated the least zone of inhibition against *F nucleatum*.

Further research, including well-designed *in vivo* and clinical studies, is necessary to validate biocompatibility, long-term antimicrobial activity, and potential translational applications of these in endodontic therapy.

CONCLUSION

From this study, it can be concluded that the use of liquorice oil, Arimedadi oil and dashmool oil as a solvent in calcium hydroxide increases antibacterial activity against *F. nucleatum*. The combination of medicinal materials is highly advantageous because each brings its own strengths and weaknesses. It is essential to utilise various antibacterial agents separately to effectively prevent any antagonistic effects that could compromise their antibacterial activities. This approach ensures optimal results in treatment and enhances overall effectiveness. Further research is needed to create effective medications that enhance antimicrobial properties and prevent bacterial recolonisation in root canal therapy.

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