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A Comparative evaluation of antimicrobial efficacy of Zingiber officinale, Allium sativum and Illicium verum extract against Enterococcus faecalis- An In Vitro study

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Abstract:

Purpose: The Purpose of this study was to evaluate and compare the antimicrobial efficacy of *Zingiber officinale*, *Allium sativum*, and *Illicium verum* extract against *E. faecalis*.

Materials and methods: Standard strains of *E. faecalis* were cultured at 10^8 CFU/mL and inoculated onto 20 Mueller–Hinton agar plates and allocated into 4 groups(n=20):-Group 1 - *Allium sativum*, Group 2 - *Zingiber officinale*, Group 3 - *Illicium verum* and Group 4 – *Normal Saline;* were assessed using the agar well diffusion method, Zones of inhibition were measured after incubation at 37°C for 24–48 hours. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.

Results: All herbal extracts demonstrated significant antimicrobial activity against both microorganisms ($p < 0.05$). Group 3 (*Illicium verum*) showed the largest mean zones of inhibition against *E. faecalis* (28.849 ± 3.595 mm), followed by Group 1 (*Allium sativum)* and Group 2 (*Zingiber officinale*) with mean Zone of inhibition is (23.527± 2.464 mm) and 22.997 ± 2.771 mm while Group 4 (Normal Saline) exhibited no antimicrobial effect.

Conclusion: Within the limitations of this in vitro study, all tested herbal extracts were effective against *E. faecalis. Illicium verum* demonstrated superior antimicrobial activity and may serve as a promising herbal alternative for intracanal irrigation.

Keywords: Zone of inhibition, *Allium sativum*, *Zingiber officinale*, *Illicium verum*, root canal infection, *Enterococcus faecalis.*

INTRODUCTION

Modern dentistry has undergone a significant transformation over recent decades, shifting from a predominantly extraction-based approach to one that emphasizes tooth preservation and functional rehabilitation. Contemporary clinical practice prioritizes the conservation of teeth affected by dental caries rather than their removal. A major advancement supporting this paradigm shift is the development of root canal therapy, which has revolutionized dental care by enabling the retention of natural teeth that would otherwise be lost.1

Root canal infections are polymicrobial in nature, with more than 600 microbial species identified within endodontic biofilms. Among these, *Enterococcus faecalis* is one of the most frequently isolated pathogens, particularly in retreatment cases and chronic apical periodontitis, with reported prevalence rates ranging from 24% to 77% as these microorganisms are notoriously difficult to eliminate due to their resistance to harsh environmental conditions and their diverse virulence mechanisms. It is a Gram- positive, facultative anaerobic coccus known for its remarkable adaptability. It can survive in both aerobic and anaerobic environments, withstand nutrient deprivation, tolerate high salt concentrations, and persist in highly alkaline conditions with pH values reaching up to 11.5, largely due to its proton pump mechanisms. This organism can penetrate dentinal tubules, adhere to collagen, form resilient biofilms, and suppress host immune responses by inhibiting lymphocyte activation.

Effective disinfection of the root canal system is essential for the long-term success of endodontic therapy. An ideal irrigating solution should possess strong antimicrobial properties, dissolve necrotic tissue, remove the smear layer, act as a lubricant during instrumentation, and be biocompatible with periapical tissues. Commonly used chemical irrigants include sodium hypochlorite (NaOCl), chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), and normal saline. Owing to the drawbacks and adverse effects of conventional irrigating agents, there has been a growing shift toward exploring herbal alternatives as potential root canal irrigants because of their antibacterial, anti-inflammatory, and antioxidant properties, along with their cost-effectiveness, environmental friendliness, and its tendency to reduce microbial load.1

Zingiber officinale (ginger) is a medicinal plant from the Zingiberaceae family, widely grown in Asia and valued for its antioxidant, antimicrobial, and anti-inflammatory properties while *Allium sativum* (garlic), belonging to the Liliaceae family, has long been used in traditional medicine because of its broad-spectrum antibacterial, antiviral, and anti-inflammatory activities. *Illicium verum* (star anise), a plant native to tropical and subtropical regions of Asia, demonstrates notable antibacterial and anti-inflammatory effects, largely due to the presence of the bioactive compound anethole.

Zingiber officinale, Allium sativum and *Illicium verum* are medicinal plants known for their antimicrobial properties. While ginger and garlic have been extensively studied, limited evidence exists regarding the antimicrobial efficacy of star anise in endodontics.

Therefore, the present in vitro study aimed to evaluate and compare the antimicrobial efficacy of extracts of *Zingiber officinale, Allium sativum*, and *Illicium verum* against *Enterococcus faecalis*.

The null hypothesis proposed that these herbal agents would not exhibit antimicrobial activity against the selected microorganism.

MATERIALS AND METHODS:

This in-vitro study was conducted in Department of Conservative Dentistry & Endodontics, Darshan Dental College and Hospital, Udaipur and at Apex Enviro Laboratory, Udaipur (Rajasthan) India.

The standard strains of *Enterococcus faecalis* (NCIM 5025) were obtained from the National collection of industrial microorganisms (NCIM) National Chemical Laboratory, Pune. Cultures were grown overnight in nutrient broth and adjusted to 0.5 turbidity on McFarland's scale. The powdered extracts of *Zingiber officinale* (ginger), *Allium sativum* (garlic) and *Illicium verum* (Star anise) were taken from Pure origanio Pvt Ltd, (India). Aqueous extract was prepared by heating ten grams of dried powder in 400 ml distilled water for six hours at low heat. After 2 hours, it was filtered through muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and was concentrated to make the final volume one-fourth of the original volume and autoclaved at 121°C and 15 lbs pressure.

Study design

In vitro antimicrobial activities of the tested herbal extracts against resistant endodontic pathogen (*E. faecalis*) were determined using the agar well diffusion method. In this study, a total of twenty Mueller–Hinton agar plates (n-20) were prepared. Aliquots of the *Enterococcus faecalis* bacterial suspension were uniformly spread on each agar plate using an L-shaped spreader. Subsequently, four wells of 6 mm diameter were prepared at equidistant points on each of the twenty agar plates using a sterile borer. Each plate was divided into four sections corresponding to the test materials. All test materials were freshly prepared and aseptically dispensed into the respective wells, ensuring that each plate contained all four tested materials. The following groups were prepared for the study:

Group 1 (n-20)- *Allium Sativum* (Garlic), Group 2 (n-20)- *Zingiber Officinale* (Ginger), Group 3 (n-20)- *Illicium Verum* (Star-anise) and Group 4 (n-20)- Normal saline.

After 24-48 hours of incubation, well defined zones of inhibition were observed. The zone of inhibition refers to the size of the clear circular zone without bacterial growth around the wells. The diameter of inhibition zones around each well was measured in millimeter using a digital vernier caliper. Three Measurements were taken for microorganism in each plate, and all measured values were registered for statistical analysis to determine the difference between the groups. Descriptive (Mean ± SD) and comparative statistics were used to compare and illustrate the results. One Way Analysis of Variance (ANOVA) followed by Tukey's Post Hoc Test was done with SPSS 16.0 software. The confidence interval was taken as 95% for the analysis ($P < 0.05$).

RESULTS

The organic extract of Group 3 (*Illicium verum)* showed the maximum zone of inhibition, followed by Group 1 (*Allium sativum*) and Group 2 (*Zingiber officinale*) against

E. faecalis. No zone of inhibition was shown by Group 4 (Normal saline). The results are shown in Table 1, Table 2, Table 3 and photograph 1. Graphic representation is shown in graph 1.
When compared statistically, all the tested natural herbal extracts showed a statistically significant antimicrobial activity against *E. faecalis* (P < 0.05).
On intra-group comparison for antimicrobial activity against *E. faecalis*, *Illicium verum* extract showed a significant (P = 0.000) and noticeably better antimicrobial activity when compared with *Allium sativum* and *Zingiber Officinale*, respectively. However, *Allium sativum* and *Zingiber officinale* extracts have comparable statistically nonsignificant (P = 0.915) antimicrobial activity.

Table 1: Descriptive analysis of mean zones of inhibition of all the irrigants against *Enterococcus faecalis*.

Groups	Sample size(N)	Mean	SD	SD error
Group 1 (2.5% A. Sativum)	20	23.527	2.464	0.551
Group 2 (2.5% Z. officinale)	20	22.997	2.771	0.620
Group 3 (2.5% I. verum)	20	28.849	3.595	0.804
Group 4 (Normal Saline)	20	0.000	0.000	0.000

Table 2: One-way ANOVA showing comparison of mean zones of inhibition of all the irrigants against *Enterococcus faecalis.*

	Sum of Squares	Df	Mean Square	F	Sig
Between Groups	9887.402	3	3295.801	494.266	0.000
Within Groups	506.773	76	6.668		
Total	10394.176	79			

Graph 1:Bar graph depicting mean zone of inhibition indicating antimicrobial efficacy of tested irrigants against E. *Faecalis*

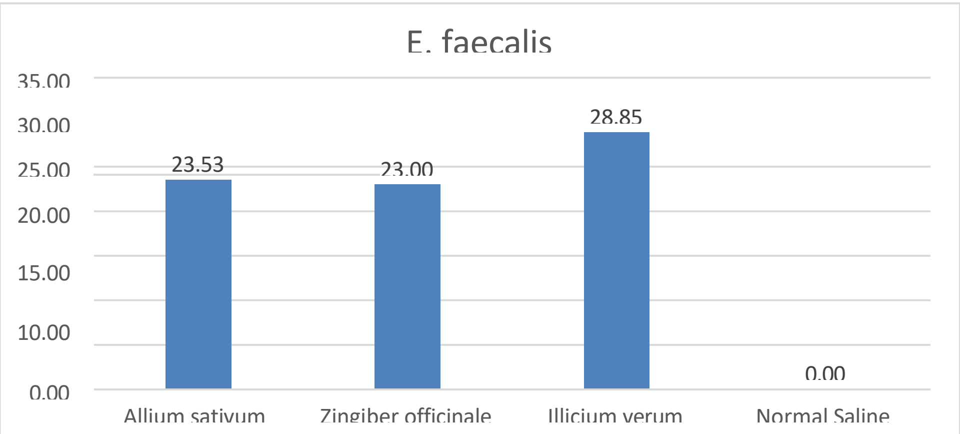
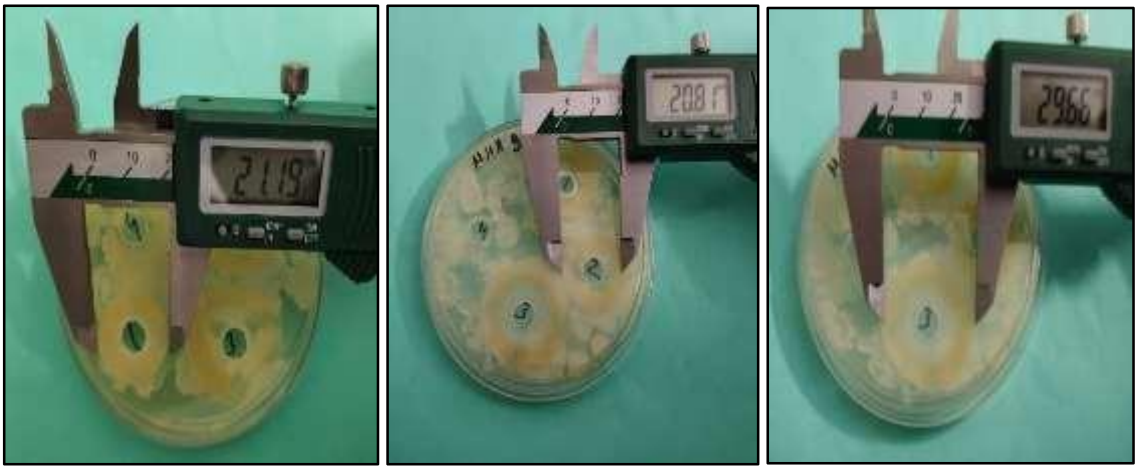


Table 3: Post hoc Tukeys analysis depicting intragroup comparison all the irrigants against *Enterococcus Faecalis*

(I) Subgroup		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Group 1 (Allium sativum)	Group 2 (Zingiber officinale)	.53050	0.817	0.915	-1.614	2.675
	Group 3 (Illicium verum)	-5.32200*	0.817	0.000	-7.467	-3.177
	Group 4 (Normal Saline)	23.52700*	0.817	0.000	21.382	25.672
Group 2 (Zingiber officinale)	Group 1 (Allium sativum)	-.53050	0.817	0.915	-2.675	1.614
	Group 3 (Illicium verum)	-5.85250*	0.817	0.000	-7.997	-3.708
	Group 4 (Normal Saline)	22.99650*	0.817	0.000	20.852	25.141
Group 3 (Illicium verum)	Group 1 (Allium sativum)	5.32200*	0.817	0.000	3.177	7.467
	Group 2 (Zingiber officinale)	5.85250*	0.817	0.000	3.708	7.997
	Group 4 (Normal Saline)	28.84900*	0.817	0.000	26.704	30.994
Group 4 (Normal Saline)	Group 1 (Allium sativum)	-23.52700*	0.817	0.000	-25.672	-21.382
	Group 2 (Zingiber officinale)	-22.99650*	0.817	0.000	-25.141	-20.852
	Group 3 (Illicium verum)	-28.84900*	0.817	0.000	-30.994	-26.704

*. The mean difference is significant at the 0.05 level.



Photograph 1: Measurement of the inhibition zones in MHA by digital vernier caliper:
(A) 2.5% *Allium Sativum*, (B) 2.5% *Zingiber officinale* and (C) 2.5% *Illicium Verum.*

DISCUSSION

Endodontic infections originate from a highly complex and diverse microbial ecosystem. The primary objective of endodontic therapy is the complete elimination of these microorganisms from the root canal system to prevent persistent infection and ensure long-term treatment success. Among the microorganisms frequently isolated from infected and failed root canals, *Enterococcus faecalis* has been strongly associated with endodontic treatment failure and recurrent periapical disease.2

It possesses exceptional survival capabilities that allow it to persist under adverse conditions such as nutrient deprivation, extreme pH levels, and exposure to intracanal medicaments. These characteristics contribute significantly to their role in persistent infections and the development of chronic apical periodontitis. Studies have reported that E. faecalis is one of the most prevalent species detected in both extraradicular infections and cases of persistent endodontic infection. Importantly, this organism rarely exists in isolation.

Owing to its marked persistence, virulence potential, and resistance to conventional intracanal procedures, this microorganism was selected for in vitro antimicrobial evaluation. Accordingly, a standard strain of *Enterococcus faecalis* (NCIM 5025) was employed in the present study to assess the antimicrobial efficacy of the tested agents.

Among the various laboratory techniques available, the agar well diffusion method is one of the most employed for antimicrobial testing. Its popularity is attributed to its simplicity, reproducibility, cost-effectiveness, and minimal equipment requirements. This method allows direct comparison of multiple antimicrobial agents against the same microorganism under standardized conditions. The formation of distinct zones of inhibition provides clear visual evidence of antimicrobial activity, which can be accurately measured using a ruler or digital caliper.12

However, the agar well diffusion method has certain limitations. It is suitable only for substances capable of diffusing through agar media, making it less effective for agents with poor solubility or limited diffusibility. Additionally, zone size does not always correlate with minimum inhibitory concentration, providing primarily qualitative or semi-quantitative data. Variables such as agar composition, medium thickness, inoculum density, pH, and incubation, conditions can also influence the results.12

Although mechanical debridement is essential in root canal therapy, chemical irrigation remains the cornerstone of root canal disinfection. Sodium hypochlorite has long been considered the gold standard irrigant due to its strong antimicrobial and tissue-dissolving properties. Despite these advantages, sodium hypochlorite has several drawbacks, including unpleasant taste, cytotoxicity to periapical tissues, risk of severe inflammatory reactions following apical extrusion, and negative effects on the mechanical properties of dentin.13

These limitations, combined with the global rise in antibiotic resistance emphasized by the World Health Organization, have renewed interest in natural, plant-derived antimicrobial alternatives. India's rich heritage of herbal medicine has contributed significantly to growing research interest in phytochemicals for dental and endodontic applications. Herbal irrigants are known for their antimicrobial, anti-inflammatory, antioxidant, and antiseptic properties, along with favorable biocompatibility, cost-effectiveness, and reduced risk of microbial resistance.

Zingiber officinale (ginger) has been valued for over 4000 years for its medicinal properties. The antimicrobial activity of ginger is attributed primarily to its phenolic compounds, including gingerols, shogaols, and paradols. along with other bioactive constituents such as quercetin and zingerone. Studies have demonstrated significant antimicrobial efficacy of ginger derivatives, particularly against *E. faecalis*.14

Allium sativum (garlic) is another extensively studied medicinal plant known for its antibacterial, antifungal, antiviral, and anti-inflammatory properties.

The primary bioactive compound, allicin, is formed when garlic is crushed and exhibits potent antimicrobial activity by interfering with vital microbial enzymes and disrupting cell membrane integrity.

Illicium verum (star anise) has gained attention for its antimicrobial, anti-inflammatory, and anti-quorum sensing properties. Its principal bioactive component, anethole, along with other phytochemicals, disrupts bacterial membranes, inhibits respiration, interferes with iron transport, and suppresses quorum sensing mechanisms essential for biofilm formation. Several studies have reported significant antibacterial and antifungal activity of Illicium verum extracts against drug-resistant pathogens.16

In the present study, aqueous extracts of garlic, ginger, and star anise were evaluated to mimic their natural mode of consumption. Aqueous extraction was selected based on evidence that water-based extracts can exhibit antimicrobial activity comparable to organic solvent extracts. Powdered herbal extracts with high purity were used to ensure standardization and reproducibility.

Among the tested herbal agents, *Illicium verum* demonstrated the highest antimicrobial efficacy, exhibiting the largest zones of inhibition against both *Enterococcus faecalis* . This enhanced activity is attributed to its rich content of phenolic compounds and flavonoids that interfere with quorum sensing and biofilm formation. *Allium sativum* and *Zingiber officinale* also showed notable antimicrobial activity, though to a lesser extent. Normal saline showed no antimicrobial effect and served as a negative control.

The null hypothesis was rejected, as all tested herbal irrigants exhibited antimicrobial efficacy against the selected microorganism. These findings highlight the clinical potential of herbal irrigants, particularly *Illicium verum*, as effective and biocompatible alternatives for endodontic disinfection and warrant further biochemical and clinical investigations.

CONCLUSION

It is possible to draw the following conclusions based on the results of this study that *Illicium Verum* extract possesses strong antimicrobial activity against *Enterococcus Faecalis,* suggesting its potential as a root canal irrigant or therapeutic agent. *Allium sativum* and *Zingiber Officinale* also showed antimicrobial effects though less pronounced compared to *Illicium Verum.* However, due to the study's limitations their clinical application in infected human canals remains uncertain. Further clinical trials are necessary to confirm their definitive role in endodontic treatment.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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