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EVALUATION OF ANTI-BACTERIAL EFFICACY OF GINGER, RESIN GUM, AND NIGELLA SATIVA IRRIGANTS AGAINST ENTEROCOCCUS FAECALIS: AN IN-VITRO STUDY

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Objectives: To evaluate the antibacterial efficacy of Ginger, Resin Gum, and Nigella Sativa, against *Enterococcus Faecalis*.

Methods: Thirty-eight extracted single-rooted premolars were inoculated with *E.faecalis,* except the negative control, and incubated for 4 weeks. After instrumentation samples were divided into 4 groups (n=7) according to final irrigation: NaOCl+EDTA (Group A), Nigella sativa (B), Resin Gum (C), Ginger (D), plus 2 control groups (n=5): Saline (E), and Saline without bacterial inoculation (F). Cultures were collected at baseline and after irrigation.

Results: One-way ANOVA showed that in groups A-D a statistically significant reduction of bacterial load was observed at T1 after final chemo-mechanical instrumentation (pNigella Sativa(99.89%), followed by ginger(99.85%) and resin gum(99.64%).

Conclusions: Herbal irrigants can be used as adjuncts to NaOCl in eradicating *E.faecalis*.

Keywords: Enterococcus Faecalis, Invitro study, Sodium Hypochlorite, Edetic Acid, Nigella sativa, Resin Gum, Ginger

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ÉVALUATION DE L'EFFICACITÉ ANTIBACTÉRIENNE DES IRRIGANTS À BASE DE GINGEMBRE, DE GOMME DE RÉSINE ET DE NIGELLA SATIVA CONTRE ENTEROCOCCUS FAECALIS: UNE ÉTUDE IN VITRO

Objectifs: Évaluer l'efficacité antibactérienne du gingembre, de la gomme de résine et de Nigella Sativa contre Enterococcus Faecalis.

Méthodes: Trente-huit prémolaires monoracinaires extraites ont été inoculées avec E. faecalis, à l'exception du témoin négatif, et incubées pendant 4 semaines. Après l'instrumentation, les échantillons ont été répartis en 4 groupes (n = 7) selon l'irrigation finale : NaOCl + EDTA (groupe A), Nigella sativa (B), gomme de résine (C), gingembre (D), plus 2 groupes témoins (n = 5) : solution saline (E) et solution saline sans inoculation bactérienne (F). Les cultures ont été collectées au départ et après l'irrigation.

Résultats: ANOVA à un facteur (PRésultats : Dans les groupes A à D, une réduction statistiquement significative de la charge bactérienne a été observée à T1 après l'instrumentation chimio-mécanique finale (pNigella sativa (99,89 %), suivie par le gingembre (99,85 %) et la gomme de résine (99,64 %)).

Conclusions: Les irrigants à base de plantes peuvent être utilisés en complément du NaOCl pour éradiquer E. faecalis.

Mots-clés: Enterococcus faecalis, Étude in vitro, Hypochlorite de sodium, Acide édétique, Nigella sativa, Gomme de résine, Gingembre

Introduction

The root canal system is a complex and variable microenvironment with an intricate network of isthmuses, accessory canals, and ramifications which may harbor various bacterial species [1]. Bacterial invasion into the root canal system, apically or coronally, produces metabolic wastes which initiate an inflammatory response [2]. Successful endodontic therapy relies on a golden triad: shaping, cleaning, and sealing the root canal system hermetically in three-dimensions [3]there has been universal agreement that the triad for endodontic success is shaping canals, cleaning in 3 dimensions, and filling root canal systems. Further, it is globally accepted that 3-D disinfection is central to success and has traditionally required a well-shaped canal. Yet, the concept of minimally invasive endodontics (MIE. The root canal system must be shaped to a sufficient apical diameter size to mechanically remove the infected pulp tissue and to serve as an effective reservoir for the delivery of the irrigating solutions [4–6].

Enterococcus faecalis *(E. faecalis)* is a facultative anaerobic, gram-positive bacterium with several shapes: single, pairs, short chains, or clusters. *E. faecalis* is one of the most resilient endodontic bacteria, it can survive and proliferate in a wide array of harsh temperatures and media [7]. It is characterized with high lipid and fatty acid content in its membrane which allows it to tolerate temperature fluctuations. Moreover, it contains bile salts, enzymes, and specific bacterial membrane configuration allowing it to survive extreme pH [8]compared to the same parameters in heat-resistant cells, remained low after an additional culture at 43-47°C, indicating a persistent effect of culture at 17-22°C. In cells grown at 10-13°C, these parameters were as low as they were in the heat-sensitive cells, provided the growth media contained an ammonium salt (1%. One of the many reasons responsible for endodontic failure is persistent infection (predominantly due to improper eradication of *E. faecalis*) [9].

Chemical irrigation is considered the basis of any endodontic therapy; it is an adjuvant tool for total eradication of microbes [10]. An ideal endodontic irrigating solution should exhibit adequate biological (non-toxic, non-carcinogenic), chemical (dissolve organic and inorganic matter...), and physical properties (optimal pH for tissue dissolution, low viscosity for enhanced penetration) [5, 11]. Sodium hypochlorite (NaOCl) (0.5-6%) is the most frequently used endodontic irrigation solution. However, it is considered toxic, has a foul taste, doesn't remove the smear layer, and might cause life-threatening complications [12]. The dual challenges of bacterial resistance to chemotherapeutics and their inherent host toxicity, researchers are interested in exploring alternative irrigants to sodium hypochlorite (NaOCl) in dental procedures, especially in endodontics [13, 14].

Ethylenediaminetetraacetic acid (EDTA) is chelating agent used as an adjunct final irrigating solution before the final rinse of NaOCl [11, 15]. EDTA 17%, composed of ethylene diamine and 4 carboxyl groups, dissolves the inorganic matter of the smear layer together with the debris that clog the dentinal tubules (calcium ions from dentin) [16].

Herbal and natural medicine have been used in dental and medical practices for centuries for their antimicrobial, antioxidant, and anti-inflammatory properties [14]. According to the World Health Organization (WHO), "Herbal medicine includes herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants, other plant materials, or combination as active ingredients." *Nigella Sativa*, Resin Gum, Zingiber Officinale (ginger), were studied for their potential antibacterial properties in eradicating endodontic related bacteria [12].

Nigella sativa, also known as black seed or black cumin, boasts a rich history in traditional medicine and has a range of potential health benefits [12]. Thymoquinone, the most abundant and potent bioactive component in the essential oil, responsible for its antibacterial, antifungal, antioxidant, anti-inflammatory, and antiviral effects [14, 17]. It has exhibited effectiveness against *Streptococcus mutans*, *Enterococcus faecalis*, and Candida albicans [18].

Ginger, a widespread spice and medicinal herb, a member of the *Zingiber Officinale* family, has been classified as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA). Its rhizome have antibacterial properties against both gram positive and negative strains, similar to Nigella sativa [17, 19, 20]. Ginger extracts showed antimicrobial efficacy against *E. faecalis* during biomechanical preparation of extracted teeth, with results comparable to 2.5% NaOCl and 2% Chlorhexidine [13]. Its major active components (gingerols and shogaols), exhibit an array of pharmacological effects in aqueous or ethanolic based solutions (5-10% concentration). These include antibacterial, antioxidant, anti-inflammatory, anti-lipid, antidiabetic, analgesic, antipyretic, and anticancer effects [21].

Resin gum or Gum Arabic Tree commonly known as *Babool-Acacia Nilotica* used for its diverse medicinal properties including antimicrobial, antioxidant, antifungal, antiviral, antibiotic, anticancer, anti-tumor, antiscorbutic, astringent, antioxidant, antispasmodic, diuretic, and anti-diarrheal activities [12, 22]. These effects are attributed to its rich composition of tannins, phenolic compounds, essential oils, and flavonoids [23]. *Babool* was used to treat conditions such as malaria,

sore throat, and toothache. Studies have confirmed its antibacterial activity against *Streptococcus mutans* and *Enterococcus faecalis* [24].

To the best of the authors' knowledge, no studies directly assessed and compared the efficacy of *Nigella Sativa*, Ginger, and Resin Gum against *E. faecalis.* This study aims to evaluate the antimicrobial efficacy of *Nigella Sativa*, Ginger, and Resin Gum in comparison to NaOCl + EDTA against *E. faecalis*.

The null hypothesis (H_0) states that there is no significant difference in the reduction of *Enterococcus faecalis* count among *Nigella sativa*, ginger, or resin gum compared to sodium hypochlorite with ethylenediaminetetraacetic acid.

Materials and Methods

This *in-vitro* study was conducted after the Institutional Review Board's approval (2023-H-0128-D-R-0566). The sample size was determined using G-power 3.1.9.7 (Germany) software tool to be at least 28 samples at a 95% confidence level and a significance level of $p = 0.05$ [25].

A double-blind design was implemented, whereby the first operator was blinded to the irrigant's identity through solution preparation and masking of syringes by a second operator. Microbiological samples were randomly coded by the second operator, prior to laboratory analysis to minimize bias.

Teeth Selection

Thirty-eight freshly extracted single-rooted human mandibular premolars with single canal anatomy (type I root canal) and complete root formation were included in this study. Mandibular premolars with multiple roots, resorptive defects, immature apices, fractures, more than one canal, or apical foramen wider than 0.15mm were excluded.

Enterococcus Faecalis Preparation

Enterococcus Faecalis (ATC 29212 American type Culture Collection) was cultured in tryptone yeast extract broth with glucose 1%, starch 2%, meat extract 0.1%, yeast extract 0.3%, and calcium carbonate ($CaCO_3$) at a neutral medium (pH=7). The enterococci grow in tryptone broth, turning the pH indicator from violet to yellow-brown color. Colonies of *E. faecalis* grow and mature between 10 and 45 degrees Celsius and range from 1-1.5 mm in diameter after incubation for 18 to 24 hours.

Herbs Preparation

Zingiber Officinale (Ginger): Aqueous ginger extract (~ 8%) was prepared by cutting 20g of ginger into thin slices and boiling it with 250 ml of distilled water. The solution was covered until cool. The extract was filtered with Whatman paper (Grade 1) then stored in an air-tight container until further use.

Nigella Sativa: In this study, we have used Nigella Sativa extract oil 100% (Hemani, Pakistan). It was prepared from freshly collected *Nigella sativa* seeds that were mechanically pressed under a low temperature (<40°C) to preserve heat sensitive bioactive ingredients of the essential oil. The oil released was then collected, filtered, and stored at a temperature of 4°C in a dark container to protect it from light and oxidation. The *Nigella sativa* extract oil was tested for purity by Gas chromatography-mass spectroscopy (GC-MS) yielding a 100% pure result with a dark yellow color and a strong aroma.

Resin Gum: 5g of Arabic gum was placed into a jar containing one cup (250 ml) of water. The aqueous mixture was stirred thoroughly to ensure a homogeneous mixture. The jar was carefully placed in a saucepan filled with hot water (not reaching the boiling temperature). After stirring and dissolving the gum, the jar was removed from the heat source and let stand overnight. The extracted aqueous solution (2%) was then stored in an air-tight container until further use.

Teeth Preparation

The mandibular premolars were decapitated to standardize their length at 16 mm. The canals were instrumented to the appropriate working length with a stainless-steel K-file size #15. Then they were autoclaved at 121°C for 20 minutes and transmitted into sterile vials each containing 50 ml of Tryptic Soy Broth (Soybean Casein Digest Broth). Ten microliters (10 ml) of *E. faecalis* were inoculated into the canals of all the teeth, except the ones of negative control, then incubated for a period of 4 weeks in brain heart infusion broth (BHI). BHI broth was renewed weekly to allow the growth of *E. faecalis* in the incubator. After 4 weeks, sterile absorbent paper points were soaked and retained in the root canals for 1 min then cultures were sent to the lab (T0) followed with full mechanical instrumentation to an apical size of 25/.06 (RevoS, MicroMega), 2 ml of 5% sodium hypochlorite (NaOCl) was used between instruments over 30 seconds. To confirm complete instrumentation, a gutta-percha cone size 25/.06 was inserted into the working length. Teeth received 3 ml of final irrigation for 1 minute as per group [9,26]. After that, another culture by means of sterile absorbent paper points reaching the working length were taken from the canals (T1).

Teeth Grouping and Sampling

The thirty-eight teeth were divided into four primary groups, 7 teeth each (n=7), and two control groups, each consisting of 5 teeth (n=5).

Group A (n=7): The irrigation solution used is NaOCl + EDTA.

Group B (n=7): The irrigation solution used is Nigella Sativa.

Group C (n=7): The irrigation solution used is Resin Gum.

Group D (n=7): The irrigation solution used is Ginger.

Group E (n=5): The irrigation solution used is Saline.

Group F (n=5): The irrigation solution used is saline and no bacterial inoculation was performed.

Microbiological Analysis

Paper points (T0 and T1) were transferred to sterile Eppendorf tubes containing 0.5 mL reduced transport fluid (RTF) and sent to the laboratory for culture and bacterial count. Colony-forming units per milliliter (CFU/mL) were determined after serial 10-fold dilutions, 10 μL of each dilution was cultured on blood agar by spread plate method. The plates were incubated aerobically overnight and proper counting was done. The percentage of bacterial count change was calculated according to Eq. (1).

Eq.(1) *Bacterial Count Change (%)* $= \left(\frac{T0\text{-}T1}{T0}\right) \times 100$

Statistical Analysis

IBM SPSS 25 program (New York, USA) for Windows was used for data entry and statistical analysis (mean and standard errors were computed). The significance level was established at $p=0.05$ and a 95% confidence interval was applied. Data is described statistically in terms of mean and standard deviation. Inferential statistics for evaluating and comparing treatment groups were performed using one-way ANOVA at baseline (T0) and after irrigation (T1). Effect size was reported as eta squared ($\eta^2 = 0.29$), indicating a large magnitude difference between groups. Duncan's Multiple Range Test (DMRT) was used post hoc to rank the six groups by their mean performance at 0.05 level and provide pairwise comparisons. Pearson's and Spearman's correlation tests at 0.05 were performed to assess the relationship between time points and bacterial count.

Results

ANOVA test and DMRT

The bacterial count of *E. faecalis* in Table 1 and Figure 1 is presented as the mean and standard deviation at both T0 and T1 in addition to the change of bacterial count (%) and confidence interval expressed at 95% confidence. The average *E. faecalis* bacterial count in T0 was recorded for 6 different treatment groups presenting various natural extracts from group A to group F: Group A NaOCl + EDTA (297.2 ± 74.3), Group B *Nigella Sativa* (3.2 ± 2.4), Group C Resin Gum (6.4 ± 1.8), Group D Ginger (1.7 ± 1.7), Group E Saline (2.0 ± 1.0), and Group F Saline without bacterial inoculation (0.0 ± 0.0). The difference between bacterial counts in T0 was non-significant as revealed by one-way ANOVA (p=0.471).

According to Table 1 the average *E. faecalis* bacterial count in T1 was recorded for 6 different treatment groups presenting various natural extracts from group 1 to group 6 was: Group A NaOCl + EDTA (0.23 ± 0.05), Group B Nigella Sativa (0.29 ± 0.50), Group C Resin Gum (1.2 ± 0.9), Group D Ginger (0.06 ± 0.10), Group E Saline (400.0 ±120.0), and Group F Saline without bacterial inoculation (0.0 ± 0.0).

The difference between bacterial count in T1 was highly significant as revealed by one-way ANOVA (p<0.001). The average *E. faecalis* bacterial count change from T0 to T1 in group A to group F was (-99.99±0.01) for NaOCl + EDTA, (-99.89±0.23) for Nigella Sativa, (-99.85±0.20) for Resin Gum, (-99.85±0.38) for Ginger , (190.00±35.00) for Saline, and (0.0 ± 0.0) for Saline without bacterial

Table 1. Bacterial count of E. faecalis pre- (T0), post treatment (T1), and percentage change for each group. Values are presented as mean ± standard deviation with 95% confidence intervals in parentheses. The significant level was set at p = 0.05 with 95% confidence level

Treatment Group	Bacterial Count of E. faecalis		
	T0 Value 10^5 (Confidence interval 95%)	T1 Value 10^3 (Confidence interval 95%)	Percentage Change %
A: NaOCl + EDTA	297.2 ± 74.3 [a] (228.5-365.9)	0.23 ± 0.05 [b] (0.18-0.28)	-99.99 ± 0.01 [c] (-100, -99.98)
B: Nigella Sativa	3.2 ± 2.4 [a] (1.0-5.4)	0.29 ± 0.50 [b] (0.05-0.75)	-99.89 ± 0.23 [c] (-100.1, -99.68)
C: Resin Gum	6.4 ± 1.8 [a] (4.7-8.1)	1.2 ± 0.9 [b] (0.4-2)	-99.85 ± 0.20 [c'] (-100.04, -99.66)
D: Ginger	1.7 ± 1.7 [a] (0.1-3.3)	0.06 ± 0.10 [b] (0.01-0.15)	-99.85 ± 0.38 [c] (-100.2, -99.5)
E: Saline	2.0 ± 1.0 [a] (0.8-3.2)	400.0 ± 120.0 [a] (251-549)	190.00 ± 35.00 [a] (146.5, 233.5)
F: Saline without bacterial inoculation	0.0 ± 0.0 [b] (0.0-0.0)	0.0 ± 0.0 [b] (0.0-0.0)	0.0 ± 0.0 [b] (0.0-0.0)
ANOVA (p-value)	0.471	<0.001***	<0.001***

[a,b,c] Means followed by different letters are significantly different according to DMRTs at 0.05 level. *, **, *** significant at p<0.05, <0.01, <0.001; ns, non-significant at p>0.05.

inoculation; respectively. The difference between the groups in change (%) of the bacterial count was highly significant as revealed by one-way ANOVA ($p<0.001$).

The confidence interval (CI) was calculated at a 95% confidence level to provide a range within which the true means is expected to lie for each group. The narrowest CI was exhibited in Group A followed by Groups B, C, and D (Table 1).

DMRTs (Figure 1) confirmed that groups A-D showed a highly significant reduction in *E. faecalis* count (>99%) over the control group. Labeled bars (a, b, and c) in Figure 1 represent the significant differences at 0.05 level. Group E (Saline only) showed an increase in bacterial count.

Pearson's and Spearman's correlation tests

After assessment of Pearson's and Spearman's correlation tests, groups A to D showed a significant negative correlation between times of observation and *E. faecalis* count. According to Spearman's correlation test (Table 2) Nigella Sativa and Ginger showed the highest inverse (negative) significant correlation with time (rs= -0.89). However, according to Pearson's correlation test (Table 2), Resin gum showed the most significant negative correlation with time (r= -0.866), followed by Nigella Sativa (r= -0.705), and Ginger.

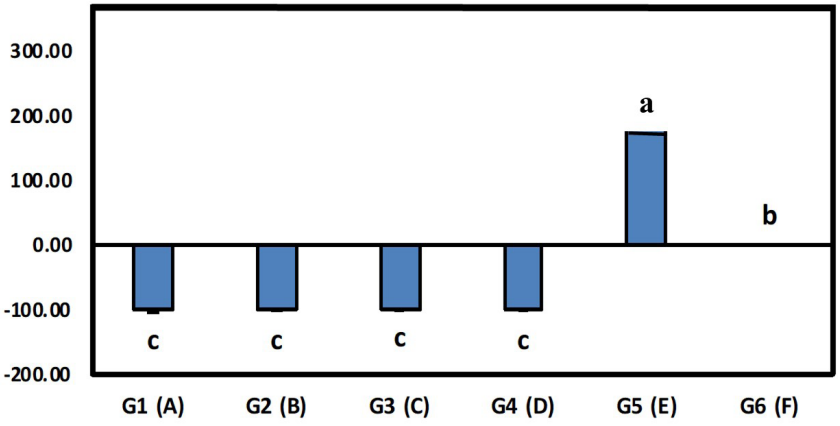

Figure 1. Bar chart presenting the percentage change of *E. faecalis* count following irrigation: mean ± standard deviation with significant groupings (DMRT)

Table 2. Spearman's and Pearson's Correlation of coefficient between time of cultures (T0 and T1) and E. faecalis bacterial count

Treatment Group	Bacterial Count at T0 and T1		
	Spearman's Correlation	Pearson's Correlation	Significance (2-tailed)
A: NaOCl + EDTA	-0.85	-0.450	<.001***
B: Nigella Sativa	-0.89	-0.705	<.001***
C: Resin Gum	-0.88	-0.866	<.001***
D: Ginger	-0.89	-0.593	<.001***
E: Saline	0.67	0.450	>0.05 ns
F: Saline without bacterial inoculation	---	---	---

Discussion

The success of endodontic therapy depends on several factors, starting from the preoperative assessment, proper diagnosis and treatment planning, complete chemo-mechanical eradication of irritating or infectious structure, ensuring a hermetic apico-coronal seal, to post-operative care [27]. Inadequate debridement creates a conductive environment for microbial persistence, facilitating bacterial colonization, proliferation, and maturation into a more resilient and complex biofilm [28, 29].

The null hypothesis (H_0), that no significant difference exists in E. faecalis reduction between Nigella sativa, ginger, resin gum, and NaOCl with EDTA was rejected ($p < 0.05$).

E. faecalis is the most frequent bacterial species in root canal-treated teeth with periradicular lesions. It can infiltrate the root canal system at various stages, intra-treatment, inter-appointment, or even post-treatment [30]. *E. faecalis* is one of the resilient bacteria, it endures prolonged periods of nutrient scarcity, with high binding affinity to dentin [2]. Furthermore, it can alter the host's immune responses, reduce the effectiveness of lymphocytes, produce lytic enzymes, utilize serum as a nutritional source, and exhibit resistance to intracanal medicaments [7, 30]. These characteristics render its complete elimination a challenging task.

Sodium hypochlorite (NaOCl) and EDTA are the most used irrigating solutions in endodontics [11]. NaOCl is valued for its ability to remove the organic components of the pulp (>2.5% NaOCl), its antibacterial properties, and its capacity to dissolve necrotic tissues [5,16]. Its efficacy depends on the concentration (0.5-8.25%), amount, and temperature used [11]. Berber et al. evaluated the effectiveness of different concentrations of sodium hypochlorite: 0.5%, 2.5%, and 5.25%, as intracanal irrigants against *E. faecalis*. The 5.25% NaOCl concentration was the most

effective, followed by the 2.5% concentration [31]. While EDTA 17% dissolves the inorganic components and removes the smear layer, created by mechanical debridement, which allows adequate penetration of NaOCl into the dentinal tubules [16]. Group A (NaOCl + EDTA) had near-total *E. faecalis* count reduction while being highly consistent in comparison to other groups. However, due to the cytotoxic and irritating effects of both solutions, especially with increased volumes, there is a continuous search for alternative irrigation solutions.

Nigella Sativa, originates from the Ranunculaceae family, contains TQ, Thymoquinone as the major bioactive component [17]. It prevents the formation of bacterial biofilms by reducing the metabolic oxidative activity of *E. faecalis* and inhibiting cell adhesion to surfaces [32]. Jain et al. compared the antibacterial potency of *Nigella Sativa* and 2.5% NaOCl against *E. Faecalis* with regards to the minimum inhibitory concentration (MIC) and mean kill time. *Nigella sativa* oil outperformed NaOCl, exhibiting a lower MIC and achieving bactericidal effects in a shorter period of time (30min and 2 hours respectively) [33]. Similarly, Group B (*Nigella Sativa*) was the second most effective solution used in decreasing *E. faecalis.*

Ginger is a constituent used worldwide in culinary and medicine, containing bioactive compounds such as volatile oils, gingerols, and shogaols [19]. In this study, ginger displayed a notable antibacterial activity in reducing *E. faecalis* count. While slower acting than *Nigella Sativa*, ginger's efficacy against resistant strains highlights its potential as an adjunct endodontic irrigant. Mokhtari et al. evaluated the antibacterial effect of 5.25% NaOCl, 2% Chlorhexidine (CHX), chloroform extract of marjoram, and oil extract of ginger. Results indicated that 5.25% NaOCl and 2% CHX exhibited the highest antibacterial efficacy compared to marjoram and ginger extracts. However, herbal groups had a low percentage of bacterial colony formation (<1%) [34]. Azhar et al. determined that the use of 15.625 mg/ml of *Zingiber Officinale Roscoe* extract had a similar effect as chlorhexidine against *E. faecalis.* Ginger's phenolic and bioactive components exhibit bacteriostatic and bactericidal effects, which are soluble in lipids and can break the phospholipid membrane of the bacteria [35].

Resin Gum or *Acacia Nilotica* is a versatile plant, utilized for the treatment of various diseases. It contains tannins, phenolic compounds, essential oils, and flavonoids, all contributing to its antimicrobial, antioxidant, antifungal, antiviral, and antibiotic properties [19]. In this in-vitro study, resin gum had a moderate antibacterial effect with slight inconsistency when compared to other herbal irrigants (*Nigella Sativa* and Ginger) against *E. faecalis.* According to Khan et al., this plant demonstrates potent antimicrobial effects against Streptococcus mutans and *E. faecalis* [36]. Another in-vitro study found that *Acacia Nilotica* antibacterial's effect against *E. faecalis* is due to the complex composition (phenolics, condensed tannins and phlobatannin, gallic acid, protocatechuic acid, pyrocatechol, catechin, epigallocatechin-7-gallate, epigallocatechin-5, 7-gallate, epicatechin, dicatechin, quercetin, leucocyanidin gallate, sucrose and catechin-5-gallate) [33].

While herbal irrigants show promising results, a major drawback lies in their variability in composition, stability, and necessity of fresh preparation to ensure efficacy. The antibacterial and antioxidant efficacy of these agents is affected by storage conditions, as exposure to high temperatures, light, and oxygen can degrade bioactive components such as thymoquinone, gingerol, shogaol [17, 21, 25]. Unlike the standardized chemical irrigation solutions, herbal extract's efficacy can be altered according to the plant source (species), extraction method, operator handling, and type of solvent utilized [25, 37].

Saline solution (Sodium Chloride, NaCl) is the control group in this study, which served as the baseline for assessing the antimicrobial efficacy of other irrigants. While saline is excellent for tissue biocompatibility it has no antimicrobial activity, this aligns with the increased bacterial counts after mechanical instrumentation in the control group (Group E). Mechanical preparation alone can't adequately reduce the microbial load in root canal systems. Endodontic files can only contact 60-70% of the root canal walls, leaving untouched areas and anatomical intricacies infected with microbial biofilm [38,39]. Jialei et al. found that instrumented dentinal walls have higher adherence affinity to *E. faecalis* than un-instrumented walls. After mechanical instrumentation the surface roughness and contact angles of the dentinal walls are altered, rendering it more prone to bacterial invasion [40]. As such, chemical disinfection is indispensable for effective bacterial eradication. All herbal irrigants tested in this in vitro study served as complementary agents to mechanical instrumentation, targeting residual microbes in inaccessible canal intricacies and untouched areas. Nonetheless, further investigations are needed to quantify their exact ability to penetrate dentinal tubules and to establish a standardized protocol to optimize their efficacy.

While this study confirms the antibacterial efficacy of ginger, *Nigella sativa*, and resin gum (>99% reduction of bacterial count), their pharmacological potential extends beyond that. These herbal irrigants are characterized by a potent antioxidant effect, which can decrease periapical inflammation through scavenging reactive oxygen species and downregulating the pro-inflammatory mediators [17, 18, 21, 24]. Moreover, bioactive constituents such as thymoquinone and gingerol can inhibit cyclooxygenase-2 (COX-

2) explaining their analgesic effects [17, 21, 34, 41]. To fully validate and understand these therapeutic effects in endodontics, it is critical to bridge the gap between in vitro and in vivo studies, ensuring comprehensive evaluation and efficacy.

Within the limitations of this study, the efficiency of herbal irrigants might be dependent and evaluating multiple concentrations would be deemed necessary to confirm it. Future research should be explored into combining different herbal agents to obtain a synergistic effect on both organic and inorganic components. Moreover, the minimum inhibitory concentration of each herbal irrigant should be quantified along with the cytotoxicity assay (biocompatibility) and tissue dissolution activity (organic and inorganic) prior to clinical application.

Conclusion

The choice of irrigating solution is critical for endodontic success. Sodium hypochlorite and Ethylenediaminetetraacetic acid have a superior antibacterial effect, even against the most resistant endodontic bacteria. Herbal irrigants as *Nigella Sativa*, Ginger, and Resin Gum exhibited promising antibacterial properties, advocating their potential use as adjuncts to the final conventional solutions. However, further research is needed to provide conclusive evidence and recommendations regarding the use of herbal extracts in endodontics.

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