

Antimicrobial efficacy of Herbal root canal irrigants and 3% sodium hypochlorite against *Enterococcus faecalis*: An *In Vitro* Study

Abstract :

Aim: To compare the antibacterial potency of Aqueous *Nigella sativa* extract, Aqueous Neem leaf extract, and 3% Sodium Hypochlorite. Test solution were tested against *E. faecalis* (ATCC 29212) to check for their Minimum Inhibitory Concentration (MIC) by double dilution method and Kill Time as a measure of their antimicrobial potency to be used as an intracanal irrigant. Within the limitations of the study, results shows that Aqueous *Nigella sativa* extract has a better antimicrobial effect compared to Aqueous Neem leaf extract and 3% Sodium Hypochlorite solution against *E. faecalis*.

Keywords: Herbal irrigants, Nigella sativa, Sodium hypochlorite.

INTRODUCTION:

Elimination of microorganisms from the root canal is crucial to successful endodontic treatment. Instrumentation and irrigation promote significant microbial reduction during the stages of chemomechanical preparation. However, complete eradication of intracanal infection is still unachievable by the methods currently available, and remaining microorganisms may cause reinfection of the root canal space. The most effective way to eliminate *E. faecalis* from the root canal space and dentinal tubule is by applying sodium hypochlorite or chlorhexidine in gel or liquid form. Due to the

disadvantages of these two commonly used conventional irrigating solutions. The use of herbal alternatives has become the dernier cri due to increased antibiotic resistance against chemical medications. Therefore, it is necessary to find a better alternative, which has led to the search for a herbal alternative.

Medicinal plants are the most abundant drugs for traditional medical systems, nutraceuticals, food supplements, modern medicines, and pharmaceuticals: intermediates and chemical entities used in the fabrication of drugs. According to the World Health Organization, 80% of people rely on therapeutic herbs used in traditional medicine.¹ The use of medicinal plants as medicine has increased worldwide due to drug failure, adverse reactions, cost of medications, and resistance to antimicrobials by bacteria.^{2,3} The most promising plant is *Nigella sativa*, with a rich historical and religious background. The most famous saying of the Holy Prophet is “Hold on to use of the black seeds, for it has a remedy for every illness except death,” and the word “Hold on” represents long-term use (Salem, 2005).⁴ *Nigella sativa*, belonging to the family Ranunculaceae. It is also commonly known as Black Seed or Black Cumin. The use of this plant appears to cut across an extensive list of ailments.

Neem (*A. indica*) is an age-old medical plant with proven antibacterial properties. It alters bacterial cell adhesion and inhibits the co-aggregation and colonization of the bacteria. The active components like alkaloids, terpenoids, tannins, nimbidin, nimbin, azadirachtin, etc., are effective towards anaerobic infections.⁵ The purpose of the present study, therefore, was to compare the antimicrobial potential of these herbal irrigants with that of sodium hypochlorite against *E. faecalis*.

MATERIALS AND METHODS:

E. faecalis, non-spore-forming fermentative facultative anaerobic bacteria which can invade the dentinal tubule, was selected as a test microorganism. *E. faecalis* (American Type Culture Collection [ATCC] 29212) was grown on Mueller – Hinton agar (Himedia, Pune, India), incubated at 37 °C, and adjusted to an optical density (OD₆₀₀) of 1 with sterile Mueller-Hinton broth.

Nigella sativa seed powder was obtained (IMPCOPS Chennai, India) and mixed with 1000 ml distilled water, vortexed until no more colour change occurred, and the solution was left at four °C for 24 hours in a sterile tube. This was centrifuged for 15 minutes at 3000 rpm, and the supernatant (brownish-orange in colour) was filtered through Whatman filter paper no. 4 and kept at 4°C in sterile tubes until use. (Khodair et al., 1993).⁶

Neem leaf powder was obtained from IMPCOPS Chennai, India. Neem leaf powder was wrapped in a muslin fabric and soaked in a beaker of 100 ml distilled water for the extraction process. The extract was filtered using Whatman filter paper no. 1 and kept in an amber bottle until use. Nigella sativa and Neem leaf extract dilutions are prepared in mg/ml concentrations by dissolving them with distilled water and sterilizing them, bypassing them with 0.22 µm nitrocellulose syringe filters (Moxcare). Their dilution is 100, 50, 25, and 12.5 mg per ml.

The tube dilution method determined the minimum inhibitory concentration (MIC) of the test solutions. Double dilution was made from a higher dilution of 100 mg/mL to a lower dilution in a series of test tubes. Each tube was inoculated with bacterial suspensions and incubated at 37 °C overnight. The MIC was regarded as the lowest concentration in the series of dilutions, which did not permit the growth of the susceptible bacteria. The subcultures were made from the tubes, which did not yield

any visible turbidity (growth) in the MIC assay on freshly prepared Mueller-Hinton agar plates. After 24 hours of incubation at 37 °C.

The time-kill study was done, which determined the time required to kill *E. faecalis* (ATCC 29212) by exposing the bacteria to 2ml of test solutions for 20 minutes. At regular intervals (5 minutes), a loop full of the sample was inoculated on a Mueller-Hinton agar plate, incubated at 37 °C for 24 hours, and observed for growth.⁷

RESULTS:

During the MIC investigation (Table 1) with Aqueous *Nigella sativa* extract, Aqueous Neem leaf extract, and 3% Sodium Hypochlorite solution, *E. faecalis* strains were sensitive to Aqueous *Nigella sativa* extract until the 4th dilution (12.5µl/ml). In comparison, the *E. faecalis* strains were sensitive to Aqueous Neem leaf extract until the 3rd dilution (25µl/ml) and 3% sodium hypochlorite until the 2nd dilution (50 µl/ml) only. Lower MIC values of the Aqueous *Nigella sativa* extract against *E. faecalis* species indicate its high degree of effectiveness against these pathogens compared to the other two test irrigants. The test was performed in triplicate to avoid bias in results.

The time-kill curve (Table 2) shows no growth at the 15th minute onwards for the *Nigella sativa* extract. At the same time, there was growth present until the 20 minutes for the specimens tested against 3 % sodium hypochlorite and Aqueous Neem leaf extract.

TABLE 1: Shows results of Minimum Inhibitory Concentration of test samples against *E. faecalis*

S.no	Test samples	100 µl/ml	50 µl/ml	25 µl/ml	12.5 µl/ml
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1.	3% NaOCl	S	S	R	R
	Aqueous Nigella sativa extract	S	S	S	S
	Aqueous Neem leaf extract	S	S	S	R
2.	3% NaOCl	S	S	R	R
	Aqueous Nigella sativa extract	S	S	S	S
	Aqueous Neem leaf extract	S	S	S	R
3.	3% NaOCl	S	S	R	R
	Aqueous Nigella sativa extract	S	S	S	S
	Aqueous Neem leaf extract	S	S	S	R

* S- Sensitive, R-Resistant.

TABLE 2: Shows Time Kill Curve Results

Test samples	0 MINS	05 MINS	10 MINS	15 MINS	20 MINS
3% NaOCl	G	G	G	G	NG
Aqueous Nigella sativa extract	G	G	G	NG	NG
Aqueous Neem leaf extract	G	G	G	G	NG

* G-Bacterial growth observed, NG – No growth observed.

Discussion:

Clinical microbiologists have three specific reasons to be interested in the role of antimicrobial plant extracts. The first reason is that the phytochemicals have found their way into the arsenal of physician-prescribed antimicrobial drugs. The second

reason is the scientists have realized that drug resistance has shortened the effective span of synthetic antibiotics. Thirdly, public awareness of the limited span of antibiotics, their abuse, and ill effects. This study used two herbal extracts, commonly used in gastrointestinal disorders, as *E. faecalis* is one of the microorganisms of concern in these conditions. *Enterococcus faecalis*, which is responsible for 80–90% of human enterococcal infections,⁸ is the dominant *Enterococcus* species and commonly the only species recovered from the obturated root canal.^{9,10} These facts indicate that *E. faecalis* has a pathogenic role in chronic endodontic treatment failure.

Sodium hypochlorite (NaOCl) is the most widely used irrigating solution. It was used as an irrigant in early 1919 as recommended by Coolidge. Berber et al. evaluated the efficacy of 0.5%, 2.5%, and 5.25% NaOCl as intracanal irrigants against *E. faecalis*. They concluded that 5.25% concentration was the most effective solution, followed by 2.5% concentration. High concentrations are associated with cytotoxic and caustic reactions; hence, this study used a lower concentration of 3% NaOCl.¹¹ Its antimicrobial property is proportional to the drug concentration, as well as its toxicity. With the reduced working time made possible by the advent of rotary instruments and techniques for root canal preparation, the irrigant of choice should exert its microbial activity quickly against resistant microorganisms found in the root canal and dentinal tubules, such as *E. faecalis*

Nigella sativa has several active components. Based on other studies, it can be hypothesized that the following could be the mode of action of *Nigella sativa*. Studies show that thymoquinone (TQ) is the major bioactive component. The study by Omar,

O. M shows that *Nigella sativa* significantly reduces the microbial flora of the infected root canals. TQ has antibacterial activity, and antibiotics could potentiate its activity.¹² Koudhi et al. demonstrated that TQ has antibacterial and resistance modifying activity and selective antimicrobial property that is effective against Gram-positive bacteria.¹³

Neem has been considered a potential source of many therapeutic agents. Earlier research on Neem showed that it contains various active constituents with various medicinal properties. The aqueous extract of Neem leaves had shown good therapeutic potential as an anti-hyperglycaemic agent; Neem showed more potency to eliminate *E. faecalis*. It may be due to the presence of nimbidin and nimbolide in neem, which possesses antibacterial, antioxidant, and antifungal properties causing bacterial cell lysis.¹⁴

Conclusion:

Within the limitations, it can be concluded that Aqueous *Nigella sativa* extract has a better antimicrobial effect compared to Aqueous Neem leaf extract and 3% Sodium Hypochlorite solution against *E. faecalis*. Extensive in vitro and in vivo studies are required before recommending these herbal root canal irrigants.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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