Journal of Pharmaceutical Research International

33(48A): 74-78, 2021; Article no.JPRI.77102
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Antimicrobial Efficacy of Herbal Root Canal Irrigants and 3% Sodium Hypochlorite against Enterococcus *faecalis*: An *In-vitro* Study

Shafie Ahamed[1], Vignesh Guptha Raju[2*], Madhuram Krishnamurthy[3], V. Naveen Kumar[3] and K. E. Selvendran[3]

[1]Department of Conservative Dentistry and Endodontics, Rajah Muthiah Dental College and Hospital, Tamilnadu, India.
[2]Department of Pediatric Dentistry and Preventive Dentistry, Karpaga Vinayaga Institute of Dental Sciences, Tamilnadu, India.
[3]Department of Conservative Dentistry and Endodontics, Karpaga Vinayaga Institute of Dental Sciences, Tamilnadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i48A33214
Editor(s):
(1) Giuseppe Murdaca, University of Genoa, Italy.
Reviewers:
(1) Zagrai Gavrila, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania.
(2) S. Thanuskodi, Alagappa University, India.
(3) Soroush Soltani, Universiti Putra Malaysia, Malaysia.
Complete Peer review History: https://www.sdiarticle4.com/review-history/77102

Original Research Article

Received 02 September 2021
Accepted 05 November 2021
Published 05 November 2021

ABSTRACT

Nigella sativa (kalonji) has been used since ancient times as a nutritional supplement and for treating various infections and chronic ailments. As pathogens become resistant to most drugs, kalonji can be used as an alternative compound in modern medicines. The use of herbal extracts as endodontic irrigants might be beneficial as a part of a growing trend to seek natural remedies for dental treatment.

Aim: To compare the antibacterial potency of Aqueous Nigella sativa extract, Aqueous Neem leaf extract, and 3% Sodium Hypochlorite.

Materials and Methods: Test solutions were tested against *E. faecalis* (ATCC 29212) to check for their Minimum Inhibitory Concentration (MIC) by double dilution method and Kill time to measure their antimicrobial potency to be used as an intracanal irrigant.

**Corresponding author: E-mail: vigneshguptha88@gmail.com;*

Results: The study's limitations show that Aqueous Nigella sativa extract has a better antimicrobial effect than Aqueous Neem leaf extract and 3% Sodium Hypochlorite solution against *E. faecalis*.

Keywords: Herbal irrigants; nigella sativa; sodium hypochlorite.

1. 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, trepenoids, 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.*

2. 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 ºC 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 [6].

Neem leaf powder was obtained from IMPCOPS Chennai, India. Neem leaf powder was wrapped in 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 37ºC

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 37ºC.

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 ºC for 24 hours, and observed for growth [7].

3. 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 (12.5 µl/ml). In comparison, the *E. faecalis* strains were sensitive to Aqueous Neem leaf extract until the 3rd dilution (25 µl/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.

4. 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* is responsible for 80–90% of human enterococcal infections, is the dominant Enterococcus species, and is commonly the only species recovered from the obturated root canal [8-10]. 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.*

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
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*

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]. Kouidhi 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]. Study performed by Mustafa M. [5] shows Neem yielded antibacterial activity equivalent to 2% chlorhexidine or sodium hypochlorite against *E. faecalis* [15] this is supported by a study done by Dakshita joy et al. they concluded that neem leaf extract could be used as an alternative agent in root canal disinfection [16].

5. 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.

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Arunkumar S, Muthuselvam M. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. World Journal of Agricultural Sciences. 2009;5(5):572-6.
2. Maryam AJ, Fatimah AA, Ebtesam AK, Abdulrahman AS, Ineta BE. In-vitro studies on the effect of Nigella sativa Linn., seed oil extract on Multidrug-resistant Gram-positive and Gram-negative bacteria. Journal of Medicinal Plants. 2016;4(2):195-9.
3. Goudarzi H, Azad M, Navidinia M, Goudarzi M. Antibacterial activity of Nigella sativa ethanolic extract against isolated bacteria from intensive care unit patients. Journal Tel. 2015;98:51-7223401.
4. Komal Anjum et al. Antimicrobial activity of aqueous extract, methanolic extract and oil of nigella sativa (kalonji) against gram positives and gram negatives clinical isolates. International Journal of Current Research. 2015;7(2):12294-9.
5. Mustafa M. Antibacterial Efficacy of Neem (*Azadirachta indica*) Extract against Enterococcus faecalis: An in vitro Study. J

Contemp Dent Pract. 2016;17(10):791-794.

6. Khodair A I, Hammouda F A and Abdel-Azim H. Phytochemical investigation of thymus de cassatus. L.Qutar University; Sci J. 1993;13(12): 211-213.
7. Chai WL, Hamimah H, Abdul lah M. Evaluation of Antimicrobial Efficacy of Antibiotics and Calcium Hydroxide against *Enterococcus faecal* is Biofilm in Dentine. Sains Malaysiana. 2013;42(1): 73–80.
8. Ruoff KL, De La Maza L, Murtagh MJ, Spargo JD, Ferraro MJ. Species identities of enterococci isolated from clinical specimens. Journal of Clinical Microbiology. 1990;28(3):435-7.
9. Molander A, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. International Endodontic Journal. 1998;31(1):1-7.
10. Dahlèn G, Samuelsson W, Molander A, Rei t C. Identification and antimicrobial susceptibility of enterococci isolated from the root canal. Oral Microbiology and Immunology. 2000;15:309–12.
11. Berber VB, Gomes BP, Sena NT, Vianna ME, Ferraz CC, Zaia AA, Souza-Filho FJ. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing *Enterococcus faecalis* within root canals and dentinal tubules. International Endodontic Journal. 2006;39(1):10-7.
12. Omar OM, Khattab NM, Khater DS. Nigella sativa oil as a pulp medicament for pulpotomized teeth: A histopathological evaluation. Journal of Clinical Pediatric Dentistry. 2012 ;36(4):335-41.
13. Kouidhi B, Zmantar T, Jrah H, Souiden Y, Chaieb K, Mahdouani K, Bakhrouf A. Antibacterial and resistance-modifying activities of thymoquinone against oral pathogens. Annals of Clinical Microbiology and Antimicrobials. 2011;10(1):1-7.
14. Bhuvan Shome Venigalla, Karthik Prasad L, et al. Comparison of Antibacterial Efficacy of Calcium Hydroxide with and without Addition of Herbal Medicaments against *Enterococcus faecalis*. Endodontology. 2015;27(1)38-41.
15. Mustafa M. Antibacterial Efficacy of Neem (Azadirachta indica) Extract against Enterococcus faecalis: An in vitro Study. J Contemp Dent Pract . 2016;17(10):791-794.
16. Sinha DJ, Nandha KDS, Jaiswal N, Vasudeva A, Tyagi SP, Singh UP. Antibacterial Effect of *Azadirachta indica* (Neem) or Curcuma longa (Turmeric) against *Enterococcus faecalis* Compared with that of 5% Sodium Hypochlorite or 2% Chlorhexidine in vitro. The Bulletin of Tokyo Dental College. 2017;58(2):103–9.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/77102