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Antimicrobial efficacy of Aqueous Nigella sativa, Aqueous Neem leaf extract, 3% Sodium hypochlorite, and 2% Chlorhexidine against endodontic pathogens belonging to different categories. – An in vitro Study

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Abstract

Aim: To evaluate the antimicrobial efficacy of aqueous Nigella sativa against planktonic forms of endodontic pathogens belonging to different categories.

Materials & Methods: Following irrigating solutions were employed

a. Herbal extracts (Aqueous Nigella sativa and Aqueous Neem leaf extract)
b. 3% Sodium hypochlorite (NaOCl)
c. 2% Chlorhexidine.

An agar culture plate was inoculated with three endodontic pathogens belonging to different categories; Gram-positive cocci Enterococcus faecalis (ATCC 29212), Gram − ve Pseudomonas aeruginosa (ATCC 27853) bacterial and fungal cells Candida albicans (ATCC 10231); zone of inhibition of microorganism was analyzed using well diffusion method.

Conclusion:

Within the limitations of this study, Herbal extracts (Aqueous Nigella sativa and Neem leaf extract) showed better antimicrobial activity.

Keywords: Antimicrobial activity, Endodontic irrigants, Herbal irrigants, Nigella sativa.

Introduction:

The oral cavity abides hundreds of microbial taxa that have evolved to survive in multispecies communities in this unique ecosystem. By contrast, the interior tissue of the tooth, i.e., the dental pulp, is a biologically sterile connective tissue in which any microbial infiltration is a pathogenic sign. It culminates in inflammation of the pulp tissue and subsequently pulp death and spread of inflammation/infection to the periradicular tissues. One of the most complex and heterogeneous microbial communities occurs in the oral cavity, where adhesion of planktonic microbes to a surface, either biotic or abiotic, is followed by coaggregation, growth, production of an extracellular matrix, and maturation of a sessile structure, the so-called oral biofilm. [1]

Despite the clear recognition that root canal infections are biofilm induced, treatments have been focused primarily on preparing canals to radiographically immaculate levels. At the same time, much is still desired on the debridement of these complicated root canal systems. Hence, just focusing on "canal shaping" significantly misses the goal of endodontic therapy since the present understanding of the microbial aetiopathogenesis of apical periodontitis urges for the emphasis to be focused on "canal cleaning" and chemomechanical disinfection. The ultimate objective of root canal therapy is to inhibit the formation of apical periodontitis by eliminating infected and \s/or inflammatory pulpal tissues and providing the aseptic intraradicular conditions compatible with periradicular healing if a lesion already exists.

The literature documents the presence of facultative anaerobic bacteria, such as Gram-positive cocci (Enterococcus faecalis), Gram-negative bacteria (Pseudomonas aeruginosa), as pathogenic for dental pulp conditions. Another opportunistic fungal pathogen, Candida spp, has occasionally been isolated from root canal periapical lesions, granulomas, and necrotic pulp tissue as well. [2-5] Candida albicans (C. albicans) is a fungus usually seen in 21% of primary infections and 18% of retreatment cases. Candida can survive in extreme environments by biofilm formation and using its physicochemical properties to suit the local conditions.

Root canal irrigants should possess an excellent antimicrobial property to enhance the outcome of the instrumentation procedures. Chemomechanical preparation is an essential step in endodontic disinfection. Sodium hypochlorite (NaOCl) and Chlorhexidine (CHX) is the most commonly used root canal irrigant. Researchers have explored several potential agents of natural origin in the search for novel irrigants with good biocompatibility and antimicrobial activity.

Nigella sativa; belonging to the family Ranunculaceae. It is also commonly known as Black Seed or Black Cumin. The use of its plant produce appears to cut across an extensive list of ailments. Nigella sativa products have been reported to treat diseases such as asthma, bronchitis, inflammatory diseases, and as an antifungal.[6] Neem (Azadirachta indica) has been regarded as one of India's most versatile medicinal herbs for over 2000 years, with a wide range of biological activities. It has been reported that neem extract has antibacterial action against Streptococcus mutans, Enterococcus faecalis, and Candida albicans. Furthermore, it is readily available and economical.[7]

The aim of this in vitro study was to evaluate the antimicrobial efficacy of aqueous Nigella sativa against planktonic forms of selected endodontic pathogens, belonging to different categories: Gram-positive cocci Enterococcus faecalis (ATCC 29212), Gram-negative Pseudomonas aeruginosa (ATCC 27853) bacterial and fungal cells Candida albicans (ATCC 10231); Using the agar diffusion method.

Materials and methods:

Aqueous Nigella sativa extract preparation:

The plants' samples Nigella sativa and Neem leaf powder (10g) were mixed with 100 ml of double-distilled water and boiled for 15 min at 60^0C using a water bath. After cooling, the extracts were filtered using Whatman No. 1 filter paper and stored at 4^0C for further experiments.

Antimicrobial activity was performed against bacterial and fungal pathogens by the well-diffusion method (Valgas et al., 2007).[8] The pure cultures of Pseudomonas aeruginosa, Enterococcus faecalis, and Candida albicans were maintained in nutrient agar (NA) slant (for bacteria) and potato dextrose agar (PDA) slant (for fungus) at 37^0C. The bacterial and fungal strains were grown overnight in NA and PDA liquid medium, respectively, on a rotary shaker (100 rpm) at 37^0C. The inoculum containing a microbial load of 1×10^5 CFU/ml was then applied to the respective agar plates. Wells of 3 mm diameter were punched aseptically with a sterile cork borer and further loaded with different samples CHX (2%), NaOCl (3%), aqueous Nigella sativa extract (100mg/ml), and aqueous Neem leaf extract (100mg/ml)) and 20 µl of gentamycin (positive control for bacteria) and 20 µl clotrimazole (positive

control for fungus). The plates were then incubated for 24 hr at 37^{0}C, and the zone of inhibition (ZOI; mm) appearing around the wells was measured. All assays were done in triplicate

Results :

Figure 1: Antibacterial sensitivity test – Showing zone of inhibition.

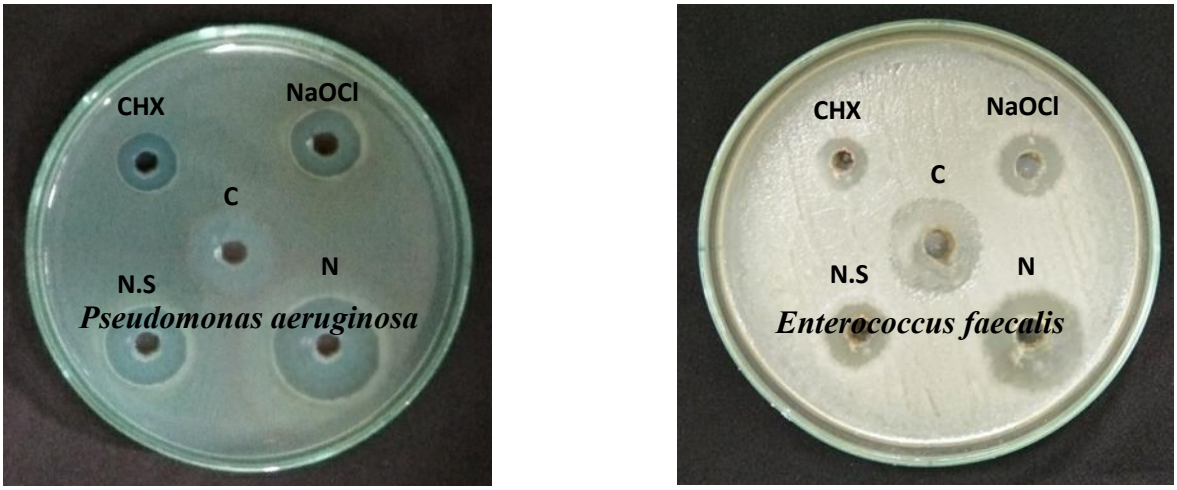

TABLE 1 Susceptibility of Pseudomonas aeruginosa and Enterococcus faecalis against test solutions.

S.No.	Name of the microorganism	Mean zone of inhibition (mm in diameter)				
		Control (Gentamicin) 20µl	CHX (2%)	NaOCl (3%)	N.sativa (100mg/ml)	Neem (100mg/ml)
1	Pseudomonas aeruginosa	20	08	10	11	22
2	Enterococcus faecalis	19	06	07	08	21

Figure 2: Antifungal sensitivity test – showing zone of inhibition.

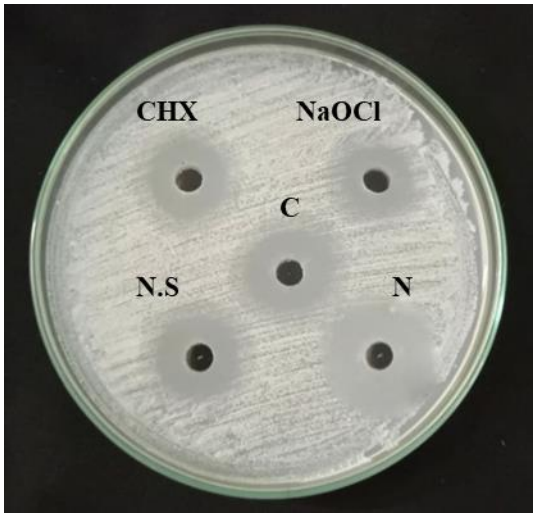

TABLE 2: Susceptibility of Candida albicans against test solutions.

S.No.	Name of the microorganism	Mean zone of inhibition (mm in diameter)				
		Control (Clotrimazole) 20µl	CHX (2%)	NaOCl (3%)	N.sativa (100mg/ml)	Neem (100mg/ml)
1	Candida albicans	21	10	11	19	23

The table 1 and 2 show the mean diameters of the inhibition zones for the tested root canal irrigant. Trials in triplicate yielded consistent results; summarizing the results, aqueous Neem leaf extract showed good antimicrobial activity followed by control, aqueous Nigella sativa extract, and 3% NaOCl. At the same time, 2 % chlorhexidine shows less activity compared to other test groups.

Figure 3: Maximum zone of inhibition shown by an irrigant as per decreasing order against endodontic pathogen

Discussion:

The increased adverse effects of contemporary medication have encouraged researchers to examine the therapeutic characteristics of novel herbals and evaluate their applicability in numerous medical science disciplines, including dentistry. Herbals employed in this study had several antibacterial, anti-inflammatory, antiviral, and antifungal effects. In dentistry, past research has assessed the irrigating capacity of herbals such as curcumin, Triphala, propolis, aloe vera, and Neem. Still, the antibacterial efficacy of Nigella sativa against pathogens belonging to different categories of root canal microbes was never assessed before this study. Nigella sativa has shown promising antimicrobial efficacy compared to NaOCl. Suppose we can extract the active thymoquinone (TQ), dithymoquinone, thymol, and thymohydroquinone, which produces antimicrobial efficacy. In that case, we can use it as a principle element in irrigation and as an intracanal medicament.

Several researchers have investigated the antimicrobial effects of different endodontic materials on various bacteria by using different techniques. The agar diffusion test we used in

this study is one of the methods most frequently used for assessing the antimicrobial activity of endodontic materials.[9] In the present study, the antimicrobial capacity of herbal root canal irrigants has been assessed using microorganisms, such as E. faecalis (Gram-positive), P. aeruginosa (Gram-negative), and C. albicans (fungus), frequently known to inhabit the human oral cavity, where they develop biofilm onto biotic and abiotic surfaces. Moreover, such microbes are among the most resistant species detectable in infected root canals, hence are typically related to endodontic treatment failures.[10]

Dutta and Kundabala analyzed the antimicrobial efficacy of five irrigants formulated from different Azadirachta indica. They compared them with 2.5% NaOCl and 0.2% CHX through an agar diffusion

test using C. albicans cultures. The authors found that NaOCl inhibited C. albicans completely.[11] The neem extract had better efficacy than CHX, which supports our study results. An in vitro evaluation of 5 different herbal extracts as endodontic irrigants against E. faecalis and C. albicans using quantitative polymerase chain reaction revealed that Neem was highly efficient to 5.25% NaOCl in reducing the counts of these microorganisms within the root canals when compared with other extracts.[12] In an experimental study by Khan et al. (2003), the aqueous extract of N. sativa seed exhibited an inhibitory effect against candidiasis in mice.[13] Finally, our results agree with Mashhadian & Rakhshandeh (2005), who showed the extract of N. sativa seeds produces antimicrobial activity against a broad range of microbes, especially on multiple antibiotic-resistant bacteria.[14] The agar diffusion test does not distinguish dental materials' microbiostatic and microbicidal properties, nor does it provide any information about the viability of the microorganisms after the test.[15] The bacteria around the inhibition zone might grow back after some days. In clinical practice, it may be possible that microorganisms could remain viable after contact with root canal irrigants; this would depend on the irrigant and its concentration.

Limitations:
However, the methodology has a limitation in that it does not simulate the clinical condition. As a result, it is implausible to extrapolate the in vitro findings to the in vivo situation. However, a comparable interpretation of this vitro data provides valuable information about the overall potential of herbal extracts.

Conclusion:
Within the limitations of this study, Herbal extracts (Aqueous Nigella sativa and Neem leaf extract) showed better antimicrobial activity.

Clinical significance:
The antimicrobial potential of Aqueous Nigella sativa and Neem leaf extract observed in this study opens new perspectives for its use as a root canal medicament and irrigating solution. The use of herbal alternatives might prove to be advantageous considering the several undesirable characteristics of NaOCl. In this study, crude extracts of all the herbals were taken. They are expected to show much more promising results if the concentrated form of principle constituents has been extracted from the corresponding herbals. Further research is needed to know their interactions with other materials and their side effects as an irrigating agent.
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Conflicts of interest: There are no conflicts of interest.

Reference:
1. Gergova R, Georgieva T, Angelov I, Mantareva V, Valkanov S, Mitov I, et al. Photodynamic therapy with water-soluble phtalocyanines against bacterial biofilms in teeth root canals. SPIE 2012; 8427: 842744
2. Hargreaves KM, Cohen S, Berman L. Cohen's pathways of the pulp. St. Louis (ed) Microbiology and Treatment of Endodontic Infections 2011. 10th ed. pp 559-600. Mosby Elsevier.
3. Valera MC, Maekawa LE, Oliveira LD, Jorge AO, Shygei É, Carvalho CA. In vitro antimicrobial activity of auxiliary chemical substances and natural extracts on Candida albicans and Enterococcus faecalis in root canals. Appl Oral Sci J 2013; 21: 118-123.
4. Carbajal Mejía JB. Antimicrobial effects of calcium hydroxide, chlorhexidine, and propolis on Enterococcus faecalis and Candida albicans. Invest Clin Dent J 2014; 5: 194-200.
5. Waltimo TM, Kuusinem M, Jarvensivu A, Nyberg P, Väänänen A, Richardson M, et al. Examination on Candida spp. in refractory periapical granulomas. Int Endod J 2003; 36: 643-647.

6. Ahamed S, Raju VG, Krishnamurthy M, Kumar VN, Selvendran KE. Antimicrobial Efficacy of Herbal Root Canal Irrigants and 3% Sodium Hypochlorite against Enterococcus faecalis: An In-vitro Study. JPRI. 2021 Nov 5;74–8.
7. Aarti Bohora. Vibha Hegde. Sharad Kokate. Comparison of the antibacterial efficiency of neem leaf extract and 2% sodium hypochlorite against E. faecalis, C. albicans and mixed culture - An in vitro study. Endontology. 2004; 6:10–14.
8. Valgas C, Souza SM, Smânia EF, Smânia Jr A. Screening methods to determine antibacterial activity of natural products. Brazilian journal of microbiology. 2007;38:369-80.
9. Carson KR, Goodell GG, McClanahan SB. Comparison of the antimicrobial activity of six irrigants on primary endodontic pathogens. J Endod 2005;31(6):471-3.
10. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008; 34: 1291-1301.
11. Dutta A, Kundabala M. Antimicrobial efficacy of endodontic irrigants from Azadirachta indica: An in vitro study. Acta Odontol Scand. 2013; 71: 1594-8.
12. Vinothkumar TS, Rubin MI, Balaji L, Kandaswamy D. In vitro evaluation of 5 different herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. J Conserv Dent. 2013; 16: 167-70.
13. Khan MA, Ashfaq MK, Zuberi HS, Zuberi AH. 2003. The in vivo anti-fungal activity of the aqueous extract from Nigella sativa seed. Phytother Res, 17: 183-186.
14. Mashhadian NV, Rakhshandeh H. Antibacterial and antifungal effects of Nigella sativa extracts against S. aureus, P. aeroginosa and C. albicans. Pakistan Journal of Medical Sciences. 2005;21(1):47-52.
15. Estrela C, Estrela CRA, Bammann LL, Pecora JD. Two methods to evaluate the antimicrobial action of calcium hydroxide paste. J Endod. 2001 Dec;27(12):720-3.