Antimicrobial Efficacy of a Novel Irrigant, *Nigella–Eugenia* Oil Composite against *Enterococcus faecalis* and *Candida albicans*

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ABSTRACT

Aim: The aim of this study was to develop a novel herbal irrigant *Nigella–Eugenia* oil composite (NEOc) and check its antimicrobial efficacy against *Enterobacter faecalis* and *Candida albicans* and compare it with standard chemical irrigants like sodium hypochlorite and chlorhexidine.

Materials and methods: Soxhlet method was chosen to extract the content from *Nigella sativa* and *Eugenia caryophyllus* herbs. Minimum inhibitory concentration (MIC)/minimal bactericidal concentration (MBC)/minimal fungicidal concentration (MFC) of *N. sativa* and *E. caryophyllus* (ethanol extracts) against *E. faecalis* and *C. albicans* was determined by well diffusion method. A novel composite NEOc was developed and its antimicrobial activity was evaluated in terms of zone of inhibition around the wells in the inoculated Mueller–Hinton agar (MHA) plates and compared with sodium hypochlorite and chlorhexidine.

Results: About 300 µg/mL of test concentrate was selected as the MIC and MBC/MFC value for the test organism for both *N. sativa* and *E. caryophyllus* extracts. Antimicrobial activity of the extracts showed good inhibitory zones. The antimicrobial activity of NEOc against *E. faecalis* and *C. albicans* was found maximum of 21 and 22 mm, respectively. The obtained results conformed the synergism behavior of two herbal extracts natively present in the developed composite. The cell viability percentage of the developed NEOc was more than 99% for all the test concentrations indicating its biocompatibility.

Conclusion: The developed novel NEOc's antimicrobial activity against *E. faecalis* and *C. albicans* was found to be slightly higher than 2.5% sodium hypochlorite and 2% chlorhexidine.

Clinical significance: The developed novel NEOc have the potential to be used as an alternative to chemical irrigants like sodium hypochlorite and chlorhexidine and decrease the incidence of endodontic treatment failures resulting from resistant bacteria like *E. faecalis* and *C. albicans*.

Keywords: *Candida albicans*, *Enterococcus faecalis*, *Eugenia caryophyllus*, irrigant, *Nigella–eugenia* oil composite.

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INTRODUCTION

The main goal of endodontic treatment is elimination of microorganisms and their byproducts from the root canal system.¹ Survival of microorganisms in the root-filled tooth is the cause of endodontic failure. According to Sjogren et al. for an effective and successful root canal treatment, the microbial infection needs to be eradicated before the root canal system is obturated.² The persistence or penetration of microorganisms into the canal at the time of obturation leads to high risk of treatment failure.³

Several studies reported the prevalence of bacteria named *Enterococcus faecalis* ranging from 24 to 77% after endodontic treatment.^{4,5} The organism is a secondary bacterial invader that resisted intracanal antimicrobial procedures and survive as a monoculture, displays persistent periapical disease in treated root canals.⁶ Stuart et al. discussed the importance of *Enterococcus faecalis* in root canal treatment failure, as well as contemporary retreatment strategies.⁷ Möller et al. studied the influence of periapical tissues of indigenous microbial flora and recovered a significant pathogen, the yeast *Candida albicans* from persistent periapical lesions of root-filled teeth.⁸ Ng also found hyphal-like structures which are suggestive of fungi in the interbacterial spaces.⁹

Endodontic infections caused by these group of organisms are currently treated by mechanical debridement along with chemical irrigants.¹⁰ Sodium hypochlorite (NaOCl) is one of the most used irrigant in endodontic therapy.¹¹ It is known for its antimicrobial action. The other functions of NaOCl include ability to partially

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neutralize necrotic tissues and completely remove pulpal remnants and pre-dentin on the instrumented surfaces.¹² Although it confers many advantageous properties, at higher concentrations it causes ulceration, hemolysis, and necrosis outside the confines of root canals and may injure periapical tissues.¹³ Other known disadvantages include unpleasant taste, tendency to bleach clothes and, inability to remove the smear layer.¹⁴ Furthermore, there are some debates about its antibacterial activity at lower concentrations, which are promoted as a way to reduce harmful reactions.¹⁵

Chlorhexidine (CHX) gluconate, a cationic bisbiguanide, is often suggested as an irrigant in endodontics for its antibacterial effects and substantivity, which permits it to reside attached to different

tissues and provide a long-lasting antibacterial activity of 8 to 12 h.¹⁶ Chlorhexidine gluconate is known to have lower cytotoxicity than NaOCl. Chlorhexidine, on the contrary, can only be used as an auxiliary irrigant or a final rinse, according to research, and is not a suitable substitute for NaOCl.¹⁷ Despite the fact that CHX is less caustic than NaOCl, it has been known to cause skin irritation.¹⁸ Furthermore, its ability to inhibit the regeneration capability of periapical tissues may be shown by its cytotoxic effects on human osteoblasts.¹⁹

As all the chemical root canal irrigants have some limitations, the search for an ideal root canal irrigant still continues. Use of traditional herbal medicines in the past has shown antimicrobial properties, together with their nontoxic nature and lesser cost they can be an ideal root canal irrigant of choice. Thus, the aim of developing nontoxic herbal irrigants with antimicrobial activity against the endodontic microorganisms is highly significant.

Nigella sativa is an annual herb of the Ranunculaceae family also called as black seed or black cumin which grows in countries bordering the Mediterranean Sea, Pakistan, and India. The black cumin seeds contain many pharmacologically active constituents like thymoquinone (TQ), dithymoquinone, thymol, and thymohydroquinone.²⁰

Eugenia caryophyllus are native to the Maluku Islands in Indonesia. Its common name is clove, commercially harvested in India, Indonesia, Sri Lanka, and Africa.²¹ The clove bud oil consists of eugenol, beta-caryophyllene, rhamnetin, crategolic acid, kaempferol, and eugenitin.²² These compounds have wide range of medicinal value that attributes in the treatment of dental diseases.

No published studies have assessed antimicrobial effect of combination of *N. sativa* and *E. caryophyllus* against *E. faecalis* and *C. albicans*. Hence in the present research, the aim is to combine *N. sativa* and *E. caryophyllus* and make a novel natural irrigant called *Nigella*–*Eugenia* oil composite (NEOc) and check its antimicrobial effect on *E. faecalis* and *C. albicans* and compare against standard root canal irrigants like 2% CHX and 2.5% NaOCl.

MATERIALS AND METHODS

The current study was conducted at Ramakrishna College of Arts and Science in Coimbatore, Tamil Nadu, India, in the Department of Microbiology. The work was completed between December 2018 and March 2020.

Collection of *Nigella sativum* and *Eugenia caryophyllus*

N. sativum (Fig. 1A) and *E. caryophyllus* (Fig. 1B) were collected from the farmhouse, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The *Nigella* seeds and *Eugenia* cloves were prewashed in deionized water and kept under shade until completely dried for milling.

Sample Preparation—Drying and Milling

N. sativa dried seeds (100 g) and 100 g of cloves were arranged separately in aluminum trays and oven dried at 40 to 50°C for 2 h until the moisture content reach <10%. The dried parts were grounded to powder and sieved using metal sieve. Sieved particles were stored at room temperature prior to testing.

Procurement of *Enterococcus faecalis* and *Candida albicans*

The challenge test organisms (*E. faecalis* and *C. albicans*) were procured from Microbiological Laboratory, Coimbatore, Tamil Nadu, India.

Soxhlet Extraction—*Nigella sativum* and *Eugenia caryophyllus*

For the present study, Soxhlet method which follows the principle of infusion method was chosen to extract the content from the given herbs. Finely milled *N. sativum* powder was placed in a porous bag or “thimble” which was made from cellulose paper and placed in the thimble chamber of the Soxhlet apparatus in the Soxhlet extraction procedure. The extraction solvent was heated in the bottom flask, and vaporized in the sample thimble, and condensed in the condenser before dripping back into the flask. The liquid content was drained back into the bottom flask when it reaches the siphon arm, and the procedure was repeated. In the Soxhlet extractor, powdered *N. sativum* plants were placed in a thimble. The extractor was filled with an ethanol solvent solution, and the temperature was set at 60°C and maintained for 6 hours.

Slowly and steadily the temperature was increased up to 100°C. The extract from the thimble was collected in the round bottom flask, kept in the heating mantle below by passing through a side arm tube. Similar experimental set up was carried out for *E. caryophyllus* powdered samples. *N. sativa* (50 mL) and 50 mL of *E. caryophyllus* was extracted separately and taken in a separating funnel and stored at room temperature prior to testing.

Figs 1A and B: (A) *Nigella sativa* (NS), (B) *Eugenia caryophyllus* (EC)

Minimal Inhibitory Concentration, Minimal Bactericidal and Fungicidal Concentration

Minimum inhibitory concentration (MIC) of *N. sativa* and *E. caryophyllus* (ethanol extracts) was determined by standard agar well diffusion method. The test organisms *E. faecalis* and *C. albicans* were grown in 5 mL of sterile nutrient broth (composition g/L: peptone: 5 g; yeast extract: 5 g, beef extract: 3 g, sodium chloride: 5 g; Final pH - 7.0 ± 0.2) for 24 h at 37°C . The growth of the organism was measured based on the turbidity in the test culture tubes. The turbidity of microbial suspension was compared and adjusted to 0.5 on the Mc Farland scale. To determine the MIC of *N. sativa* and *E. caryophyllus*, a set of Mueller–Hinton broth tubes (five tubes) were prepared under sterile conditions for each test organism. About 100 μL of extracted *N. sativa* and 100 μL *E. caryophyllus* separately at different concentrations (100, 200, 300, 400, and 500 $\mu\text{g/mL}$) was added to each Mueller–Hinton broth tubes. Followed by about 10 μL of test culture suspension (*E. faecalis* and *C. albicans*) was added to the broth separately and observed for turbid growth. All the tubes were incubated for 24 h at 37°C . The lowest dilution inhibiting the growth of test organism was selected as the MIC of *N. sativa* and *E. caryophyllus*.

To determine the minimal bactericidal and fungicidal concentration (MBC/MFC) of the *N. sativa* and *E. caryophyllus* extracts, a loopful of sample was taken from the selected MIC tube of respective organism and inoculated onto Mueller–Hinton agar (MHA) plates (composition g/L acid hydrolysate of casein: 17.5 g; starch: 1.5 g, sodium chloride: 5.0 g, agar 17.0 g; final pH - 7.0 ± 0.2). All the plates were incubated and observed for the absence of growth determining the MFC and MBC of the selected herbal extracts.

Preparation of *Nigella*–*Eugenia* Oil Composite

Using *N. sativum* and *E. caryophyllus* ethanol extracts, an herbal composite (NEOc) was developed. Briefly, 10 mL of *N. sativum* extracts was kept under stirring conditions using a magnetic stirrer (180 rpm, 40°C) in a beaker. Followed by adding 10 mL of *E. caryophyllus* extracts dropwise onto the above extract at the rate of 1 mL per minute. The magnetic stirring condition was kept constant for 2 h till complete development of composite. Developed NEO composites (20 mL) were stored in brown, amber bottle at refrigeration temperature prior to antibacterial activity and other tests.

Antimicrobial Activity of Herbal Irrigants

The antimicrobial activity of *N. Sativa*, *E. caryophyllus* extracts, and NEOc was evaluated against the test organisms by well diffusion method. Two sterile MHA (composition g/L acid hydrolysate of casein: 17.5 g; starch: 1.5 g, sodium chloride: 5.0 g, agar 17.0 g; final pH (7.0 ± 0.2)) plates were prepared and allowed to solidify in one plate about 0.1% inoculum suspension of *E. faecalis* and in another plate same amount of inoculum suspension of *C. albicans* was swabbed uniformly over the agar surface. Under sterile conditions, three 6 mm wells were cut on the agar surface of each MHA plate. About 20 μL of *N. sativa*, 20 μL *E. caryophyllus* extracts and 20 μL of the developed NEO composite was loaded in the MHA plate inoculated with *E. faecalis*, similarly the MHA plate inoculated with *C. albicans* also was loaded with the same amount of the herbal extracts in three 6 mm wells and both the plates were incubated at 37°C for 24 h (Fig. 2). The antimicrobial activity was evaluated in terms of zone of inhibition around the wells in both the inoculated MHA plates. The inhibition clear zones were measured and recorded in millimeter.

Antimicrobial Activity of Chemical Irrigants

In parallel the antimicrobial activity of 2.5% NaOCl and 2% CHX was evaluated against the test organisms by well diffusion method. Two sterile MHA (composition g/L acid hydrolysate of casein: 17.5 g; starch: 1.5 g, sodium chloride: 5.0 g, agar 17.0 g; final pH (7.0 ± 0.2)) plates were prepared and allowed to solidify. About 0.1% inoculum suspensions of the test organism (*E. faecalis* and *C. albicans*) were swabbed uniformly over the agar surface separately. Under sterile conditions, 6 mm wells were cut on the agar surface of each MHA plates. About 20 μL of 2.5% NaOCl and 20 μL of 2% CHX were loaded into the well and the plates were incubated at 37°C for 24 h. The antimicrobial activity was evaluated in terms of zone of inhibition around the wells in all the inoculated MHA plates. The clear zones of inhibition were measured and reported in millimeters.

Comparison of Antimicrobial Activity of Herbal and Chemical Irrigants

The antimicrobial activity of *N. sativa*, *E. caryophyllus*, and NEOc was compared with 2.5% NaOCl and 2% CHX using the zone of inhibition values calculated against the test organisms *E. faecalis* and *C. albicans*.

Figs 2A and B: Antimicrobial activity of the *Nigella sativa* (NS), *Eugenia caryophyllus* (EC), and *Nigella-Eugenia* composite (NEOc) against (A) *Enterococcus faecalis* and (B) *Candida albicans*

In Vitro Biocompatibility of NEOc Using Standard MTT Assay Methods

Cytotoxicity assay was used to evaluate the *in vitro* cytotoxicity. L₉₂₉ fibroblast cell line used to evaluate cytotoxicity of NEOc.

To achieve confluence growth, the fibroblast cell lines were grown in 12-well microtiter plates. The composite sample was directly applied to the fibroblast monolayer that had formed. The plates were prewetted in a 70% aqueous ethanol solution for 48 hours before cell seeding, then washed twice with ultrapure water. The specimens were subsequently seeded with 10,000 cells per well of the L₉₂₉ fibroblast cell line, as per standard cell culture procedures. For 15 days, the plates were incubated at 37°C with 5% CO₂. The photometric MTT test was used to assess the influence of composite on fibroblast biocompatibility.

Samples were obtained from the 24-well plates at each time point and transferred to new plates for the MTT analysis. The MTT solution was prepared by dissolving the powder in phosphate-buffered saline at a concentration 1 mg/mL. After 1 h of incubation, the purple crystals were dissolved by adding sodium dodecylsulfate (SDS) in a 1:1 mixture of water and dimethyl formamide (DMF) at a concentration of 20% w/v. After adding 1 mL of MTT medium (0.0005 mg/mL) to each well, the plates were incubated for 3 h, rinsed and desorbed in 100 µL of 70% isopropanol. After being agitated rapidly at 400 rpm/min for 40 min, the dyed medium was transferred to 96-well plate, and read at 550 nm. The biocompatibility or cell viability is expressed as a percentage of the control sample (100%).

RESULTS

Minimal Inhibitory Concentration and Minimal Bactericidal/Fungicidal Concentration

Minimal inhibitory concentration and MBC/MFC of *N. sativa* and *E. caryophyllus* extracts were analyzed against *E. faecalis* and *C. albicans*. Among the four test concentrates of *N. sativa*, 300 µg/mL was found to be the MIC and MBC/MFC value for *E. faecalis* and *C. albicans*. In Table 1 the MIC and MBC/MFC of *N. sativa* was presented, respectively. Absence of growth in the respective tubes and plates determined the MIC and MBC/MFC values of *N. sativa* extracts against the test organisms. Similar results were found evident for *E. caryophyllus* extracts against the *E. faecalis* and

C. albicans. About 300 µg/mL of test concentrate was selected as the MIC and MBC/MFC value for the test organism. Absence of growth in the respective tubes and plates determined the MIC and MBC/MFC values of *E. caryophyllus* extracts against the test organisms (Table 2).

Antimicrobial Activity of Herbal Irrigants

The antimicrobial activity of the *N. sativa*, *E. caryophyllus*, and *Nigella*–*Eugenia* composite (NEOc) against *E. faecalis* and *C. albicans* were compared with 2.5% NaOCl and 2% CHX in the present study. The comparison was carried out to determine whether the synergistic effect of composite and the individual effect of plant extracts was significant in retarding the growth of dental pathogens in comparison with the efficacy of chemical irrigants.

About 18 and 19 mm of inhibitory zones were obtained for the ethanol extracts of *N. sativa* against *E. faecalis* and *C. albicans*. Almost similar size of inhibitory zones (17 and 19 mm) were recorded for the ethanol extracts of *E. caryophyllus* against the respective test organisms. However the antimicrobial activity of the developed NEOc was found higher in terms of inhibitory zones than the individual plant extracts. The zone of inhibition for the NEOc against *E. faecalis* was 21 mm and against *C. albicans* was 22 mm.

Antimicrobial Activity of Chemical Irrigants

Antimicrobial activity of chemical irrigants against *E. faecalis* and *C. albicans* was tested separately for 2.5% NaOCl and 2% CHX (Fig. 3). About 17 and 19 mm inhibitory zones were obtained for the irrigant 2.5% NaOCl against *E. faecalis* and *C. albicans*, respectively. Inhibitory zones of about 19 and 20 mm were found evident against the respective test organisms for the other chemical irrigant 2% CHX.

Comparison of Antimicrobial Activity between Herbal and Chemical Irrigants

The inhibitory zones of the developed NEOc were about 21 and 22 mm against *E. faecalis* and *C. albicans*, respectively. The inhibitory zones of NEOc were higher relatively when compared with the inhibitory zones of individual plant extracts and individual chemical irrigants. The comparison of antimicrobial activity of the *N. sativa*, *E. caryophyllus*, and NEOc with 2.5% NaOCl and 2% CHX is presented in Figure 4.

Table 1: Minimal inhibitory concentration of *Nigella sativa* against *Enterococcus faecalis* and *Candida albicans*

S. No.	Test organism	Presence or absence of growth				
		1	2	3	4	5
1	<i>Enterococcus faecalis</i>	+	+	–	–	–
2	<i>Candida albicans</i>	+	+	–	–	–

+ Presence of microbial growth, –Absence of microbial growth

1: 100 µg/mL, 2: 200 µg/mL, 3: 300 µg/mL, 4: 400 µg/mL, 5: 500 µg/mL

Table 2: Minimal inhibitory concentration of *Eugenia caryophyllus* against *Enterococcus faecalis* and *Candida albicans*

S. No.	Test organism	Presence or absence of growth				
		1	2	3	4	5
1	<i>Enterococcus faecalis</i>	+	+	–	–	–
2	<i>Candida albicans</i>	+	+	–	–	–

+ Presence of microbial growth, – Absence of microbial growth

1: 100 µg/mL, 2: 200 µg/mL, 3: 300 µg/mL, 4: 400 µg/mL, 5: 500 µg/mL

Figs 3A and B: Antimicrobial activity of sodium hypochlorite (SH) and chlorhexidine (CHX) against (A) *Enterococcus faecalis*; (B) *Candida albicans*

Fig. 4: Graphical representation of antimicrobial activity of herbal and chemical irrigants

In Vitro Biocompatibility of NEOc Using Standard MTT Assay Methods

The biocompatibility of the developed NEOc was investigated using standard MTT assay method. During the analysis, the cell viability percentage was more than 99% for all the test concentrations indicating the biocompatibility of the NEOc (Table 3). In addition, no cytotoxicity was evident on the developed L₉₂₉ fibroblast cells.

The biocompatibility of the developed NEOc was investigated using standard MTT assay method. As the cell viability percentage was found highly significant (>99%) for all the test concentrate, the biocompatibility of the developed composite was thus proved evident.

DISCUSSION

Bacteria such as *E. faecalis* and *C. albicans* have been associated with cases of endodontic treatment failure. the existing chemical endodontic irrigants used against these bacteria are NaOCl and CHX. Sodium hypochlorite has some disadvantages such as unpleasant taste, tendency to bleach, and soft tissue inflammation when it is passed outside the confines of root canal,²³ and CHX has cytotoxic effects on human osteoblasts when used as an irrigant, possibly indicating its ability to reduce the periapical tissues'

regeneration capability,¹⁹ and also has been reported that it causes irritation to the skin.¹⁸

The search for an ideal root canal irrigant is continued to overcome the disadvantages of the existing endodontic irrigants and remove resistant bacteria from the root canal treatment and reduce the endodontic treatment failures. Irrigants made from natural sources has the potential to be ideal root canal irrigant because of its nontoxic nature, and its limited use against the antibiotic-resistant bacteria. Hence in the present study, based on the antimicrobial activity of *N. sativa* and *E. caryophyllus* extracts, specifically against dental infections a herbal composite was developed in the present study and termed as NEOc and tested whether the individual extracts or newly developed NEOc can be used as natural irrigants to combat against infection caused by *E. faecalis* and *C. albicans*.

The combination of two different herbal drugs were selected based on the character of synergism. Synergistic activity of drugs in a combination contains potential antimicrobial activity when compared to be tested alone. Mode of action of two different drugs on targeting the cellular components of a microbe influences more antimicrobial activity than the action of a single drug. Based on this synergistic character, *N. sativa* and *E. caryophyllus* are combined as a novel composite and antimicrobial activity against dental

pathogens *E. faecalis* and *C. albicans* were studied in the present study.

Cytotoxicity assays are used commonly to evaluate the *in vitro* cytotoxicity.²⁴ The biocompatibility of the developed NEOc was investigated using standard MTT assay method. During the analysis, the cell viability percentage was more than 99% for all the test concentrations indicating the biocompatibility of the *Nigella*–*Eugenia* oil composite. In addition, no cytotoxicity was evident on the developed L₉₂₉ fibroblast cells. However, some notable differences in cell morphology and appearance of few vacuoles (Fig. 5) have been found in the lower concentrates of composites (10 and 20 µg concentrates). The appearance of these vacuoles may be due to the fusion of multiple membranes of fibroblast cells.

Minimal Inhibitory Concentration and Minimal Bactericidal/Fungicidal Concentration

Minimal inhibitory concentration and MBC/MFC of *N. sativa* and *E. caryophyllus* ethanol extracts were analyzed against *E. faecalis* and *C. albicans*. Among the four test concentrates of *N. sativa* and *E. caryophyllus*, 300 µg/mL was found to be the MIC and MBC/MFC value for *E. faecalis* and *C. albicans*. Similar works carried out by many researchers are discussed below which were found supportive to our present study.

Chaieb et al. reported the MIC value of *N. sativa* extract was found to be 32 µg/mL against *E. faecalis*.²⁵ The MIC value of ethanol extracts of *N. sativa* in the present study was found to be 300 µg/mL against *E. faecalis*. MIC value obtained in Chaieb et al.'s work was found comparatively less (32 µg/mL); this may be due to pure TQ compounds used rather than the crude extracts of *N. sativa* in the present study.

In another study, Aires et al. evaluated the bacteriostatic and bactericidal abilities of *E. caryophyllus* against oral bacteria in planktonic and biofilm states. The results obtained were promising, with best antibacterial activity against oral pathogen *E. faecalis* (MIC and MBC of 100 µg/mL),²⁶ whereas in the present research it was 300 µg/mL. The differences in the MBC and MIC values obtained in our study may be due to different factors such as methods adopted, source of *E. caryophyllus* used, and type of strains tested.

Comparison of Antimicrobial Activity between Herbal and Chemical Irrigants

The antimicrobial activities of the *N. sativa*, *E. caryophyllus*, and NEOc against *E. faecalis* and *C. albicans* were compared with NaOCl and CHX in the present study. The comparison was carried out to determine whether the synergistic effect of composite and the individual effect of plant extracts was significant in retarding the

Table 3: Cytotoxicity analysis of *Nigella*–*Eugenia* oil composite (NEOc)

Fibroblast cell lines–MTT assay			
S. No.	concentration (µg)	Cell viability (%)	Cytotoxic reactivity
1	5	> 99	No cytotoxicity
2	10	> 99	No cytotoxicity
3	15	> 99	No cytotoxicity
4	20	> 99	No cytotoxicity
5	25	> 99	No cytotoxicity

Fig. 5: Microscopic analysis of the biocompatibility of *Nigella*–*Eugenia* oil composite (NEOc) using MTT assay

growth of dental pathogens in comparison with the efficacy of chemical irrigants. The inhibitory zones of *N. sativa*, *E. caryophyllus*, and NEOc showed promising results and the composite showed relatively more activity than individual herbs.

In the present study, the MIC and MBC/MFC values of ethanol extracts of *N. sativa* was found to be 300 µg/mL against *E. faecalis* and *C. albicans*, and about 18 and 19 mm of inhibitory zones were observed for the extracts of *N. sativa* against *E. faecalis* and *C. albicans*, respectively. Jain et al. in her study found that that *N. sativa* oil has an MIC of 0.4 µL/mL against *E. faecalis*²⁷ and Hanafy and Hatem observed that mean diameter zone of inhibition in discs containing ether extract of 400 µg nigella sativa on MHA plates inoculated with *C. albicans* was 2.1 cm.²⁸ Direct comparison of MIC, MBC/MFC could not be done from these studies, because of different methodology adopted. However, the results of both the previous studies were found to be supportive to our present research in terms of inhibitory action of *N. sativa* against *E. faecalis* and *C. albicans*.

In the present study, the MIC and MBC/MFC values of ethanol extracts of *E. caryophyllus* was found to be 300 µg/mL against *E. faecalis* and *C. albicans*, and about 17 and 19 mm of inhibitory zones were observed for the extracts of *E. caryophyllus* against *E. faecalis* and *C. albicans*, respectively. Silvestri et al. found that eugenol which is the major compound responsible for the antimicrobial activity of *E. caryophyllus* showed MIC and MBC of 1 µg/mL against *E. faecalis*.²⁹ The findings of Silvestri et al. could not be compared with results of our study because of different methodology, but the findings of Silvestri et al.'s study were supportive to our present research findings in terms of antimicrobial activity of *E. caryophyllus* against *E. faecalis*.

Mohamed and Badri investigated antimicrobial activity of *E. caryophyllus* against *C. albicans* and observed 32 mm of zone of inhibition.³⁰ The study of Mohamed and Badri was supportive to the present research in terms of inhibitory action of *E. caryophyllus* against *C. albicans*. However, the difference in the zone of inhibition can be because of the source of *E. caryophyllus* or its extraction method or different strains of *C. albicans* used in the study.

The antimicrobial activity of NaOCl (17 and 19 mm) and CHX (19 and 20 mm) also revealed similar inhibitory zone size against *E. faecalis* and *C. albicans* when compared to the natural oil extracts. Jose et al. focused on the impact of microbes forming biofilm postendodontic treatment. Hence the researchers aimed to compare a commercial disinfectant, QMiX with chemical irrigants (NaOCl and CHX) and natural irrigants (guava leaf extract, and *A. vera* extract) in terms of antimicrobial activity against *E. faecalis*. During this analysis, the researchers reported that all test solutions showed good antimicrobial activity against the test organism.³¹ In terms of antibacterial activity of NaOCl and CHX against *E. faecalis*, the work of Jose et al. was found to be supportive of our current research. Bidar et al. studied the comparative antimicrobial effect of 2% CHX, 2.5% NaOCl, and MUMS containing 2% CHX against *C. albicans*. The researchers revealed that chlorhexidinated MUMS showed antimicrobial effect like CHX solution.³² In our present study also 2% CHX solution showed good antimicrobial effect in terms of zone of inhibition against *C. albicans*.

In the present research the antimicrobial activity of the developed NEOc expressed in terms of zone of inhibition was 21 and 22 mm against *E. faecalis* and *C. albicans*. The inhibition zones of NEOc were relatively higher than the individual plant extracts and the individual chemical irrigants. The combination of two different herbal drugs in the developed composite were selected based

on the character of synergism. Synergistic activity of drugs in a combination contains potential antimicrobial activity in terms of mode of action on targeting the cellular components of a microbe to make it lethal than the action of a single drug. In accordance to literature survey, the mode of action of herbal extracts was described in terms of inhibitory activity on the microbial cells. The mode of action involves the lethal effect of herbal extracts on the cell membrane of organisms; active components of extracts and their hydrophobicity enable them to partition the lipids of the cell membrane, rendering them permeable and leading to leakage of cell contents, finally leading to cell lysis.³³ The synergistic activity of two different herbal oil extracts in the developed composite was found evident in terms of antimicrobial activity when tested against *E. faecalis* and *C. albicans*.

Limitations of the Present Study

The present study is an *in vitro* study in which the conditions of the root canal has not been replicated, contact time for bactericidal action with the organisms has not been studied. Mechanism of action at cellular and molecular level should be determined and controlled preclinical and clinical trials should be conducted before NEOc can be recommended to be used alternatively with chemical irrigants during endodontic therapy.

CONCLUSION

This study highlighted the promising antimicrobial activity of a novel composite termed NEOc formulated by combining *N. sativa* and *E. caryophyllus* extracts against *E. faecalis* and *C. albicans*. *Nigella-Eugenia* oil composite exhibited maximum antimicrobial activity than the activity exerted by the herbs alone, thus conforming the synergism between two herbal extracts present in the developed composite. *Nigella-Eugenia* oil composite can be used as an alternative to chemical irrigants such as NaOCl and CHX after understanding its effectiveness in preclinical trials using simulated root canal conditions, and clinical trials with optimized parameters.

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