

ANTIBACTERIAL EFFECTIVENESS OF *NIGELLA SATIVA* IRRIGATION ON *ENTEROCOCCUS FAECALIS* BIOFILM USING PASSIVE ULTRASONIC IRRIGATION OR WATERLASE LASER. (IN-VITRO STUDY)

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ABSTRACT

Objectives: To compare the antimicrobial properties of *Nigella sativa* (N.Sativa-0.3%) and sodium hypochlorite (NaOCl-5%) root canal irrigation solutions against *Enterococcus faecalis* (*E.Faecalis*), utilizing ultrasonic, and laser activation methods in extracted teeth.

Material and methods: Seventy extracted mandibular premolars including solitary canals underwent instrumentation, sterilization, then inoculation with *Enterococcus faecalis*. Following twenty-one days of incubation, the samples were randomly divided into seven groups (n=10) including a negative control group. The tested irrigations *Nigella sativa* -0.3% and NaOCl-5% were subdivided according to the activation protocol: no activation, passive ultrasonic irrigation Irrisafe ultrasonic tips and laser-assisted irrigation (Waterlase: Er,Cr,YSGG).

The cultured dentinal shavings were got using H-files and paper points. The antibacterial activity was evaluated via bacterial colony-forming units per milliliter (CFUs/ml). Mann Whitney U test and Kruskal-Wallis H test were performed (P<0.05).

Results: Significant reduction was obtained with all tested groups except for the non-activated *Nigella sativa* -0.3%. NaOCl-5% demonstrated the significant greatest antimicrobial efficacy with each irrigation protocol. Non-significant difference existed when comparing the anti-microbial efficacy of non-activated NaOCl-5% to the laser activated *Nigella sativa* -0.3%.

Conclusions: The group of non-activated *Nigella sativa* was the unique group that failed to show a significant anti-microbial efficacy against *Enterococcus faecalis*. Sodium hypochlorite exhibits the highest statistically significant efficacy in the eradication of *Enterococcus faecalis* bacterial biofilm, disregarding the activation method used.

The laser potentiates the anti-microbial result of *Nigella sativa* irrigation to the extent of reaching the efficacy of non-activated high concentration of sodium hypochlorite solution.

KEYWORDS: *Enterococcus faecalis*, Laser, *Nigella sativa*, Ultrasonic.

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INTRODUCTION

Irrigation plays a central role in disinfecting and debriding root canals, especially within anatomical complexities that are inaccessible to instrumentation, for example: isthmuses, lateral canals, and accessory canals ⁽¹⁾. Irrigation is the sole antimicrobial agent during single-visit treatment. Various irrigating solutions serve different functions, including flushing debris, dissolving tissues, chelation, and antimicrobial action. However, many irrigating solutions have cytotoxic adverse effects when accidental periapical extrusion occurs ⁽²⁾. Currently, no single irrigating solution is considered optimal ⁽³⁾.

Sodium hypochlorite (NaOCl) has been endorsed for irrigation during chemo-mechanical preparation of root canals for its solvent and antimicrobial actions ⁽⁴⁾. High concentrations of NaOCl (5.25%) exhibit strong antimicrobial effects while being highly toxic. Conversely, low concentrations of NaOCl (0.5%) effectively dissolve necrotic tissue but show weak efficacy against bacteria ⁽⁵⁾. Sodium hypochlorite has several drawbacks as its failure to remove the smear layer alone, its foul odor, potential to cause emphysema, allergic reactions, toxic effects on surrounding tissues, as well as detrimental impacts on dentin elasticity and bending resistance ^(5,6).

Recently, there has been an increased interest in herbal irrigation alternatives due to their reduced cytotoxic side effects compared to other irrigation solutions. Achieving a balance between antimicrobial effectiveness and cytotoxicity is crucial for irrigation used in regenerative endodontics and vital pulp therapy. The use of essential oils in endodontic practice showed promise due to their high antibacterial potential and absence of resistance issues. Many herbs were reported to possess multiple beneficial properties, such as biocompatibility, anti-inflammatory, antioxidant, antimicrobial, availability, and cost-effectiveness, thus promoting the concept of “natural and green dental practice” ⁽⁷⁻⁹⁾.

Nigella sativa (*N.Sativa*) showed strong antimicrobial action when used as root canal irrigation ⁽¹⁰⁾. The seeds contain bioactive compounds such as Nigellone and thymoquinone, which exhibit powerful antimicrobial action against a broad spectrum of pathogens, including bacteria, fungi, and yeast. Studies have also shown synergistic effects with conventional antibiotics, enhancing their efficacy against resistant strains. Beyond its antimicrobial properties, *Nigella sativa* boasts diverse pharmacological activities, including anti-inflammatory, analgesic, and immunomodulatory effects. Its safety profile is favorable, with minimal reported toxicity. ^(11,12) Its cytotoxic effects were found to be directly proportional to its concentration ⁽¹³⁾.

The development of multiple techniques to enhance the efficacy of irrigation within the root canals through agitation, such as ultrasonics and lasers ^(3,14). Ultrasonic agitation has been shown to improve the dissolution of necrotic pulp tissue, the eradication of bacterial biofilms and the removal of the smear layer ⁽¹⁵⁻¹⁷⁾. During passive ultrasonic irrigation (PUI), the smooth oscillating file produces circular stream and bubbles cavitation. ⁽¹⁸⁾

However, Badami et al., 2023 systematic review demonstrated that laser-activated irrigation LAI has superior efficacy over Ultrasonic activation in the elimination of microorganisms, dentin debris, and smear layer from the root canal system ⁽¹⁴⁾. Laser was found to create expanding and imploding vapor bubbles with secondary cavitation, resulting in quick fluid motion intra or extra canal ⁽¹⁹⁾. Middle infrared lasers as erbium, chromium:yttrium-scandiumgallium-garnet (Er,Cr:YSGG) and erbium-doped yttrium aluminium garnet Er:YAG lasers have been frequently utilized for irrigation activation using the low power. The Er,Cr:YSGG laser is extremely captured by water even if existing only inside the bacteria ^(19,20). Er,Cr:YSGG laser via radial emitting tip of Waterlase (Biolase, Irvine, California, USA) has shown a significant

antimicrobial result on the infected dentine ⁽²¹⁾. Yet, the high cost and safety issues of erbium lasers reduced their use by the endodontists ⁽²⁰⁾.

Enterococcus faecalis (*E.faecalis*) is prevalent in 24% to 77% of resistant endodontic infections. This gram-positive facultative anaerobe can thrive nutritional scarce, struggle with other microorganisms, and penetrate inside the dentinal tubules ⁽²²⁾.

The effect of laser and ultrasonic activation on *Nigella sativa* extract irrigation was not previously investigated. The efficacy of activated *N.Sativa* irrigation against *E.faecalis* will be compared to one of the highest concentration of sodium hypochlorite (5 %) used in root canal disinfection.

The null hypothesis stated that no difference will be observed in the antimicrobial efficacy between the lack of activation, ultrasonic activation, and laser activation when comparing sodium hypochlorite irrigation solution and *Nigella sativa* irrigation solution in the eradication of *E.faecalis* bacterial biofilm.

AIM OF THE STUDY

The aim of this in-vitro study was to compare the antimicrobial effects of *Nigella sativa* and sodium hypochlorite root canal irrigation solutions against *E.faecalis* without activation and with ultrasonic or laser activation methods.

MATERIALS AND METHODS

Study design and sample size calculation

The current work followed the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines ⁽²³⁾ Figure (1). Ethical approval was gotten from the research ethics committee, Faculty of Dentistry (02-2024-07-01-3-229). Informed consent was written from the patients or their guardians who approved the use of their extracted teeth in the present study.



Fig. (1) PRILE 2021 Flowchart for the present study (Post-experiment).

Sample size was planned via power analysis and sample size software (PASS 2020) from NCSS, LLC. Kaysville, Utah, USA. The minimum entire hypothesized sample of 70 sampling units (10 per group) was required to assess and compare the antibacterial effectiveness of *N.Sativa* and NaOCl

with and without activation modes (ultrasonic or laser) in human teeth model against *E.faecalis*, considering a significance level of 5% α and a power of 80%⁽²⁴⁻²⁶⁾.

Teeth preparation

All teeth were placed in NaOCl - 2.5% solution for five minutes and stored in saline solution at room temperature till their use. Any residues, debris, and calcific deposits on the root surfaces were removed by curettes and pumice brushing. Digital radiographs were taken to verify the absence of calcifications, irregularities, or resorptions in root canals. Inclusion criteria included single rooted mandibular premolar with single canal and closed apex. Exclusion criteria involved canal calcification, root resorption, cracks, caries and previous root canal treatment.

The occlusal surface was then flattened with a diamond disk to produce a standardized root length of 20 mm. A stainless-steel k file size #10 was inserted beyond the apical foramen to ensure patency, after which 1 mm was subtracted for working length establishment. After conventional access cavity preparations, canals were shaped using TruNatomy Shaping Files kit Up to size #35/4 (Dentsply Maillefer, Ballaigues, Switzerland). The root canals were irrigated with 1 ml of saline solution with a 30-gauge endodontic irrigation needle between each file and after the preparation. The roots were protected externally by two layers of nail polish and the apices of all teeth were closed with a light-cured resin composite (Nexcomp, Dentsply Maillefer, Ballaigues, Switzerland).

Each tooth was solely put in a cryovial with 500 μ l of brain heart infusion (BHI) broth (HiMedia Laboratories Pvt. Ltd, India) and autoclaved at 121° C for 30 min. It was mounted vertically on silicon impression material (zeta plus condensation silicone, Zhermack, Badia Polesine, Rovigo, Italy) to receive another sterilization cycle in autoclave⁽²⁷⁾.

Preparation of *Nigella sativa* extracts (0.3% or 3 microliters/milliliters (μ l/mL))

300 microliters of *Nigella sativa* cold pressed oil (Imtenan Co, Qalyubia, Egypt) were solubilized using 900 microliters of dimethylsulfoxide (DMSO) and further diluted with sterile distilled water up to a final volume of 100 ml⁽¹³⁾.

Preparation of *E. faecalis* biofilm

Tryptone Soy Agar (TSA) sealed petri dish was used to grow *E. faecalis* (ATCC29212) at 37° C for 24 h. Then suspending the colonies within 5 ml of Tryptone Soy Broth (TSB) was performed to attain one McFarland via spectrophotometer. Injection of the root canal by the suspension of *E. faecalis* was conducted by a sterilized insulin plunger to become incubated at 37 °C for 21 days under aseptic aerobic situations. The repetition of inoculation every 3 days to guarantee the bacterial life was performed^(28,29).

Specimens' allocation and grouping

Randomization was performed using computer generated list of random numbers (<http://www.random.org/>).

The samples with coded numbers were randomly divided into three groups corresponding to the irrigation material used: Group (1): *Nigella sativa* (0.3%), Group (2): Sodium hypochlorite (5%) (JK dental vision, Mansoura, Egypt), and Group (3): Negative control group (without any irrigation). Each tested group was further divided into three subgroups based on the irrigation activation method: Subgroup (1A/2A): Irrigation without activation five milliliters for one minute. Subgroup (1B/2B): Passive ultrasonic irrigation activation using Irrisafe ultrasonic tips (Satelec Acteon, Merignac, France). This was performed for three cycles using a stainless-steel iso size 25 ultrasonic endo tips inside the canal for 20 seconds at a power setting of 5.5 W 30 kHz by piezoelectric ultrasound unit Suprasson P5 Booster



Fig. (2) Passive ultrasonic irrigation (Intra-experiment).

(Satelec Acteon, Merignac, France) ⁽³⁰⁾ Figure (2). Subgroup (1C/2C): Subgroup (1C/2C): laser-assisted irrigation activation was performed using Waterlase: Er,Cr,YSGG (epic; Biolase, Irvine, California, USA) with a power of 1.25W, air: 20, water: 20, mode H, and a pulse setting of 50Hz according to the manufacturer protocol. The tip used was RFT2-21mm (0.25 mm in diameter). The laser was activated only while withdrawing the tip at 2mm per second ⁽³¹⁾ Figure (3). Sodium thiosulfate was used to neutralize the irrigation effects, before sampling. The canal samples were gathered by only one endodontist through dentin scraps, 1 mm shorter than the working length, by Hedstrom file (H-file) #30 (MANI, Japan) for 30 s ⁽³²⁾. Dentin powder was gathered via three paper points left to the previous length 60 s. As the sampling procedure was destructive; reducing or eliminating the bacterial biofilm, the level of bacterial burden at baseline for each group before the disinfection procedures was not performed to avoid any bias. Therefore, the bacterial count was performed after 21 days of incubation before the disinfection procedures from ten randomly selected teeth (the negative control group) ⁽²⁷⁾.

The contaminated paper points and H-files were placed into a cryovial containing 1ml TSB and were vortexed for 60 s and then 10-fold serially diluted.



Fig. (3) Laser-assisted irrigation activation using Waterlase: Er,Cr,YSGG (Biolase, Irvine, California, USA) (Intra-experiment)

Sterile spreaders collected 10µL of the resultant serial dilution to be instantly plated in 4 closed TSA petri dish and incubated at 37° C for 48 h ⁽³³⁾. The Colony-forming units per milliliter (CFUs/ml) was counted per dish via a colony counter (Fisher Scientific, Waltham, MA, USA.) according to Barbosa- Ribeiro et al. (34) and Martinho and Gomes ⁽³⁵⁾.

Success was defined according to the presence of significance in discrepancies between any tested group and the control group regarding their antimicrobial efficacy Figure (4).

BLINDING

The microbiological specialist and the statistician were blind to the treatment group.

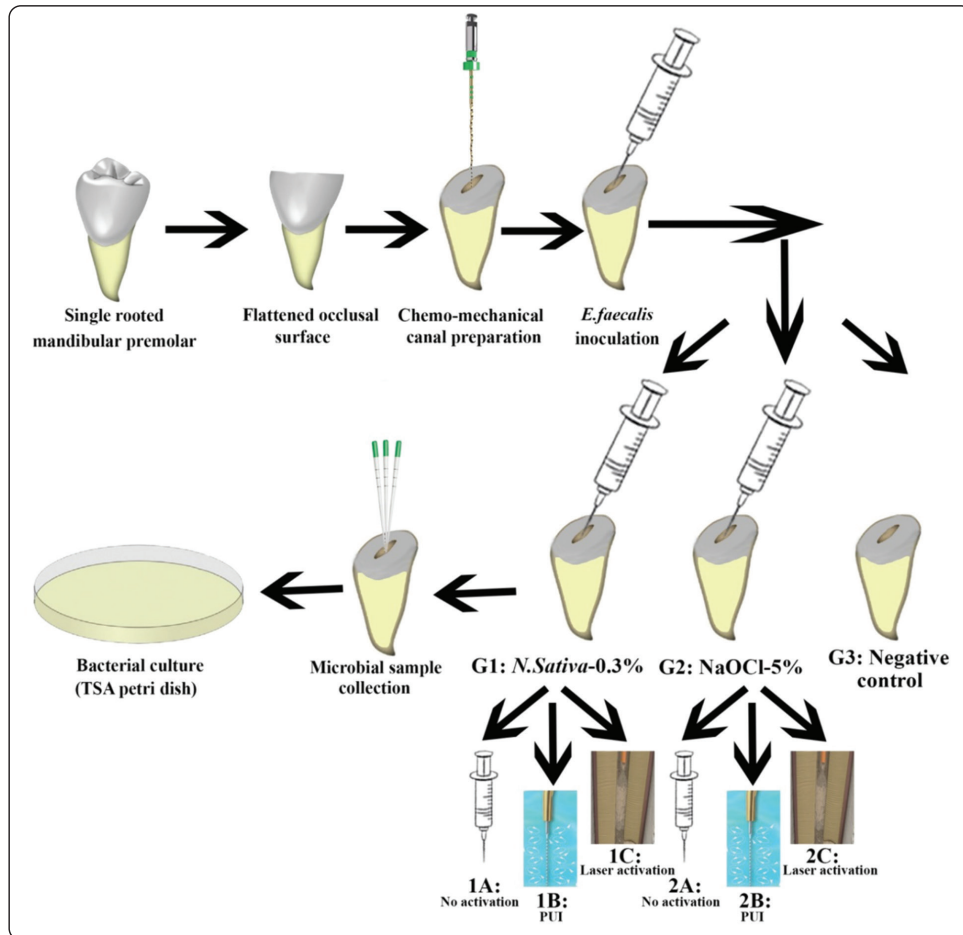


Fig. (4) Diagrammatic presentation of the sequential steps: canal instrumentation, E.faecalis inoculation, specimen groups and quantitative investigation (Pre-experiment).

Statistical analysis

Data was investigated by means of IBM SPSS software package version 29.0. (Armonk, NY: IBM CORP) and r version 4.3.2. Quantitative data was defined by mean, standard deviation, range (minimum and maximum), or median and interquartile range (IQR). Significance of the obtained results was arbitrated at the 5% level, and the confidence interval at 95%. Mann Whitney U test was utilized for non-parametric distributed quantitative variables for comparability between two categorical groups. Kruskal-Wallis H test was conducted for non-parametric distributed quantitative variables, for comparing between more than two categorical groups.

RESULTS

Successful outcome was obtained with all tested groups except the group of non-activated *Nigella sativa*, which exhibited statistically non-significant difference with the negative control group Tables (1,2) and Figures (5,6).

When sodium hypochlorite was used with or without activation, no statistically significant difference existed between the three subgroups.

Within the three subgroups of *Nigella sativa*, a highly statistically significant difference (p value = 0.009) was found between the laser activated subgroup and the non-activated subgroup, with laser showing the greatest antimicrobial efficacy. However, absence of significance was displayed

during the comparison of the ultrasonic activated subgroup with the non- activated subgroup or with the laser activated subgroup.

A statistically significant difference originates when comparing the antimicrobial effects of sodium hypochlorite with *Nigella sativa* irrigation using

each irrigation protocol, with sodium hypochlorite demonstrating the greatest antimicrobial efficacy.

Yet, a non-significant difference was seen when comparing the anti-microbial effect of non-activated sodium hypochlorite solution to the laser activated *Nigella sativa* solution.

TABLE (1) Comparison between 7 different groups of samples.

Group	1A	1B	1C	2A	2B	2C	3	Statistical test	P value
Median	54400	12800	5080	1660	70	80	126240	26.238	<0.05 ^a
(IQR)	(41620)	(76260)	(13640)	(35905)	(350)	(260)	(87720)		
(Range)	(31920- 87680)	(3800- 90400)	(320-17080)	(20- 65520)	(20- 460)	(0- 320)	(27200- 163200)		

*p value (<0.05) was considered significant using When you can apply the Kruskal-Wallis H test (a)

TABLE (2) Pairwise comparisons between the different groups of samples.

Group	Gp. (no.)	Gp. (no.)	Statistical test	P value
(3-1A)	Gp (3)	Gp (1A)		
Median (IQR)	126240 (87720)	54400 (41620)	5	0.117 ^b
Range	(27200- 163200)	(31920- 87680)		
(3- 1B)	Gp (3)	Gp (1B)		
Median (IQR)	126240 (87720)	12800 (76260)	2	0.028 ^a
Range	(27200- 163200)	(3800- 90400)		
(3- 1C)	Gp (3)	Gp (1C)		
Median (IQR)	126240 (87720)	5080 (13640)	0	0.009 ^a
Range	(27200- 163200)	(320- 17080)		
(3- 2A)	Gp (3)	Gp (2A)		
Median (IQR)	126240 (87720)	1660 (35905)	1	0.016 ^a
Range	(27200- 163200)	(20- 65520)		
(3-2B)	Gp (3)	Gp (2B)		
Median (IQR)	126240 (87720)	70(350)	0	0.009 ^a
Range	(27200- 163200)	(20- 460)		
(3- 2C)	Gp (3)	Gp (2C)		
Median (IQR)	126240 (87720)	80 (260)	0	0.009 ^a
Range	(27200- 163200)	(0- 320)		
(1A-1B)	Gp (1A)	Gp (1B)		
Median (IQR)	54400(41620)	12800(76260)	9	0.465 ^b
Range	(31920- 87680)	(3800- 90400)		

Group	Gp. (no.)	Gp. (no.)	Statistical test	P value
(1A- 1C)	Gp (1A)	Gp (1C)		
Median (IQR)	54400(41620)	5080 (13640)	0	0.009 ^a
Range	(31920- 87680)	(320- 17080)		
(1A- 2C)	Gp (1A)	Gp (2C)		
Median (IQR)	54400(41620)	80 (260)	0	0.009 ^a
Range	(31920- 87680)	(0- 320)		
(1B- 1C)	Gp (1B)	Gp (1C)		
Median (IQR)	12800 (76260)	5080 (13640)	6	0.175 ^b
Range	(3800- 90400)	(320- 17080)		
(1B- 2C)	Gp (1B)	Gp (2C)		
Median (IQR)	12800 (76260)	80 (260)	0	0.009 ^a
Range	(3800- 90400)	(0- 320)		
(2A-1A)	Gp (2A)	Gp (1A)		
Median (IQR)	1660 (35905)	54400 (41620)	3	0.047 ^a
Range	(20- 65520)	(31920- 87680)		
(2A- 1B)	Gp (2A)	Gp (1B)		
Median (IQR)	1660 (35905)	12800 (76260)	4	0.076 ^b
Range	(20- 65520)	(3800- 90400)		
(2A- 1C)	Gp (2A)	Gp (1C)		
Median (IQR)	1660 (35905)	5080 (13640)	11	0.754 ^b
Range	(20- 65520)	(320- 17080)		
(2A- 2C)	Gp (2A)	Gp (2C)		
Median (IQR)	1660 (35905)	80 (260)	4	0.076 ^b
Range	(20- 65520)	(0- 320)		
(2B-1A)	Gp (2B)	Gp (1A)		
Median (IQR)	70(350)	54400(41620)	0	0.009 ^a
Range	(20- 460)	(31920- 87680)		
(2B- 1B)	Gp (2B)	Gp (1B)		
Median (IQR)	70(350)	12800(76260)	0	0.009 ^a
Range	(20- 460)	(3800- 90400)		
(2B- 1C)	Gp (2B)	Gp (1C)		
Median (IQR)	70(350)	5080 (13640)	1.5	0.021 ^a
Range	(20- 460)	(320- 17080)		
(2B-2A)	Gp (2B)	Gp (2A)		
Median (IQR)	70 (350)	1660 (35905)	5.5	0.142 ^b
Range	(20- 460)	(20- 65520)		
(2B-2C)	Gp (2B)	Gp (2C)		
Median (IQR)	70(350)	80 (260)	10.5	0.675 ^b
Range	(20- 460)	(0- 320)		
(2C- 1C)	Gp (2C)	Gp (1C)		
Median (IQR)	80 (260)	5080 (13640)	0.5	0.012 ^a
Range	(0- 320)	(320- 17080)		

*p value (<0.05) was considered significant using When you can apply Mann Whitney U Test (a)

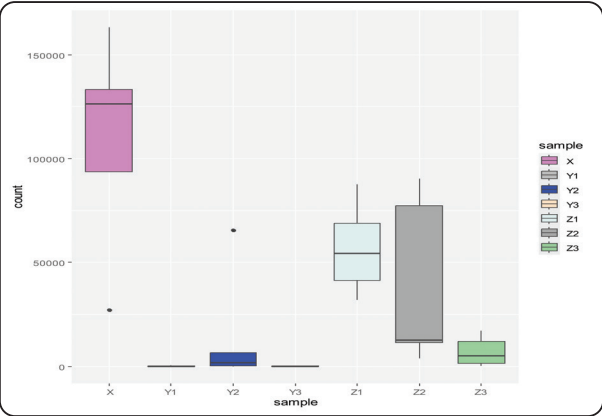


Fig. (5) Box plot illustrates the comparison between the different 7 groups of samples (Post-experiment).

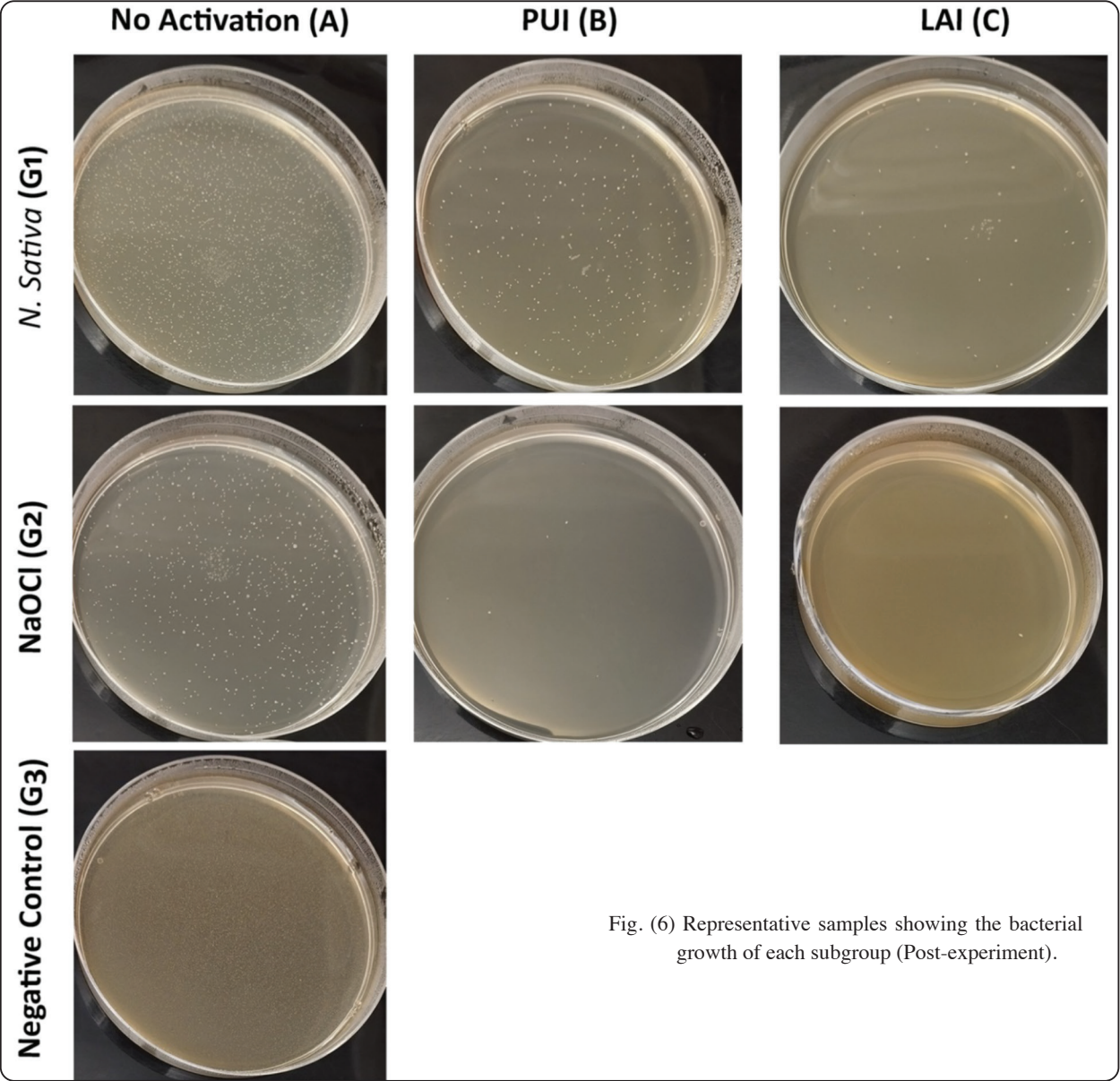


Fig. (6) Representative samples showing the bacterial growth of each subgroup (Post-experiment).

DISCUSSION

The rationale of the current work was to present an irrigation solution with minimal cytotoxic effect while achieving maximum anti-microbial efficacy. This is of great importance in case of single visit root canal treatment, vital pulp therapy and regenerative endodontic treatment. Therefore, the antimicrobial efficacy of a non-cytotoxic low concentration of *Nigella sativa* was studied with and without activation and compared to the strong antimicrobial concentration of sodium hypochlorite (5 %). Furthermore, Laser and ultrasonic activation had previously shown favorable effects on stem cells used in regenerative endodontic treatment⁽³⁶⁻³⁹⁾. The present study selected to test the anti-microbial efficacy against *E.Faecalis* biofilms, because it was found persistent in failed retreated root canals, resisting harsh conditions inside the dentinal tubules as nutritional deprivation and alkalinity⁽²²⁾. The current work used the *N.Sativa* cold pressed oil dissolved in DMSO as CLSI recommendations for water-insoluble drugs⁽⁴⁰⁾. Cold pressed oils preserving the contained pro- and anti-oxidative compounds are free from any organic solvents that might modify the minor ingredients, useful characteristics or oily stability^(41,42).

The present study utilized a low concentration of 3 μ l/mL of *N.Sativa* oil, considering the results of Ugur et al., 2016 that reported the absence of any cytotoxic effect at concentrations up to 1 μ g/mL (equivalent to 1.2 μ l/mL) on the gingival fibroblasts and also observed a minimum inhibitory concentrations (MIC) value of 2 μ g/mL (equivalent to 2.4 μ l/mL) against *E.faecalis*⁽¹³⁾.

The current work results demonstrated that the non-activated *Nigella sativa* irrigation was the unique group that failed to show a significant anti-microbial efficacy against *E.faecalis*. This was found in accordance with Al-Sheik et al 2006 showing that the aqueous extract of *Nigella sativa* did not eliminate *E. faecalis* while ethanolic extract did⁽⁴³⁾.

This disagrees with previous studies showing a strong anti-microbial effect of *Nigella sativa* irrigation^(10,13,43,44). This disagreement may be due to the higher concentration and different methodology used by these studies for evaluation of the antimicrobial efficacy as observing the minimum inhibitory concentrations tested in wells. However, the present in vitro study tried to simulate the clinical conditions by using human root canal inoculated with *E.faecalis* to detect reduction in CFU count. Furthermore, longer contact time might be needed to produce better antibacterial action as Jain et al., 2019 used *Nigella sativa* irrigation and reported that the time kill curve of *E.faecalis* had demonstrated absolutely no growth at 30 minutes of exposure⁽⁴⁴⁾. Yet, the current study left the *N.Sativa* solution inside the canal for one minute following the same recommended time for the sodium hypochlorite irrigation for standardization⁽⁴⁵⁾.

Additionally, differences may exist in oil extraction methods or percentage, and quality of the active components of the oils according to geographic locations^(46,47).

The significance resulting when comparing the antimicrobial effects of sodium hypochlorite with *Nigella sativa* irrigation using each irrigation protocol, with sodium hypochlorite demonstrating the greatest antimicrobial efficacy.

This finding may be due to the strong dissolving and antimicrobial actions of sodium hypochlorite^(4,5). Moreover, these significant differences between both irrigation solutions used may be due to the selected minimal biocompatible concentration of *Nigella sativa* and the high concentration of sodium hypochlorite used in the present study. As their anti-microbial power and cytotoxicity were found to increase with high concentrations^(5,6,13).

Both activation methods used in the current study empowered the anti-microbial efficacy of *N.Sativa* to achieve a statistically significant difference in comparison to the control group.

Interestingly, the impact of laser activation significantly enhanced the antimicrobial efficacy to the level of reaching the effect of the non-activated high concentration sodium hypochlorite (5%). This demonstrated the efficacy of laser as a valuable adjunct to *Nigella sativa* extracts in root canal disinfection. This may be attributed to laser's ability to create unstable vapor bubbles with a secondary cavitation effect that improved apical washing and smear layer elimination with the radial emitting tip of Waterlase reaching the infected dentinal tubules⁽¹⁹⁻²¹⁾. Moreover, Acoustic streaming which includes fluid speed and turbulence is the main effect achieved from ultrasonic tips. Cavitation, which is the formation of expanding imploding bubbles within the fluid is found less powerful in ultrasonics⁽⁴⁸⁻⁵¹⁾. Furthermore, the effect of laser on the constituents of *N.Sativa* oil might play a role as previously reported that the laser can be used to cleave a phenolic compound or benzene ring to simpler compounds⁽⁵²⁾. The phenolic profiles earlier analyzed by Kiralan et al., 2014 using High-performance Liquid Chromatography exposed that thymoquinone was the chief phenolic compound in which great concentrations of benzoic and *p*-hydroxy benzoic acids existed within cold pressed *N.Sativa* oil⁽⁴²⁾. The current study results regarding the effect of Waterlase laser activation showing significant superiority for NaOCl(5%) over *N.Sativa* disagrees with the results of Hadwa et al., 2023⁽⁵³⁾ who underwent a study in primary teeth and demonstrated the highest percentage of bacterial reduction with the diode-*Nigella sativa* group, without a statistically significant difference from diode-sodium hypochlorite (2.5%) using Diode laser (Lasotronix, smart M pro 635nm). This may be explained by many different factors as the type of teeth, the type and power of laser, and the concentrations of both *N.Sativa* oil and NaOCl.

No significant difference was seen in the current work between laser and ultrasonic activation on both tested irrigation solutions. This was in accordance

with Aydin et al 2020 who found beneficial effects of Er,Cr:YSGG laser and ultrasonic system against *E. faecalis* with NaOCl 2.5% (25). However, this disagreed with Race et al., 2019 (54) who showed that passive ultrasonic irrigation PUI produced a significant great decrease of microbes in the root canal compared to the waterlase, without any difference in the apical third using 4% NaOCl and 15% EDTA.

This in vitro study reveals notable understandings into the antimicrobial efficacy of *Nigella sativa* and sodium hypochlorite irrigation solutions when subjected to different activation methods.

The present study results support the growing interest in alternative herbal irrigation solutions. It contributes valuable information to the ongoing exploration of alternative irrigation solutions and activation methods. It recommends the use of laser activated low concentration *Nigella sativa* oil as an effective anti-microbial irrigation protocol.

Further investigations about the impact of laser over the different constituents of *Nigella sativa* oil are needed as well as research about the optimal concentration for *Nigella sativa* as irrigation solutions.

The limitations of the current study include the selected concentrations to be compared and the type of laser used. Under the previous limitations, the null hypothesis is rejected.

CONCLUSIONS

The group of non-activated *Nigella sativa* was the unique group that failed to show a significant anti-microbial efficacy against *E.faecalis*. Sodium hypochlorite exhibits the highest statistically significant efficacy for abolition of *Efaecalis* bacterial biofilm, disregarding the activation method used. Laser and ultrasonic activations significantly enhance the antimicrobial impact of *Nigella sativa*. The laser potentiates the anti-microbial effect of

Nigella sativa extract to the extent of reaching the efficacy of non-activated high concentration of sodium hypochlorite solution.

Abbreviations

(H-file)	Hedstrom file
CFUs/ml	Colony-forming units per milliliter
DMSO	Dimethylsulfoxide
<i>E.Faecalis</i>	<i>Enterococcus faecalis</i>
Er,Cr:YSGG	Erbium, chromium:yttrium-scandium gallium-garnet
Er:YAG	Erbium-doped yttrium aluminium garnet
Hz	Hertz
IBM SPSS	Statistical Package for the Social Sciences
kHz	Kilohertz
LAI	Laser-activated irrigation
mlm	Milliliter
mm	Millimeter
<i>N.Sativa</i>	<i>Nigella sativa</i>
NaOCl	Sodium hypochlorite
nm	Nanometer
PUI	Passive ultrasonic irrigation
TSA	Tryptone Soy Agar
TSB	Tryptone Soy Broth
W	Watt
WL	Working length
μL	Microliter

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