

## Original Research

### Naocl Vs Herbal Irrigants, With/Without Laser, Against E. Faecalis: An In-Vitro Study

<sup>1</sup>Nitin Lokhande, <sup>2</sup>Treesa Mathew, <sup>3</sup>Sathish Abraham, <sup>4</sup>Pradnya Nagmode, <sup>5</sup>Priyani Jaiswal, <sup>6</sup>Tejas Patil

<sup>1-6</sup>Department of Conservative Dentistry and Endodontics, SMBT Dental College, Sangamner, Maharashtra, India

#### ABSTRACT:

This research endeavours to evaluate the potential of herbal alternatives to supplant sodium hypochlorite (NaOCl), the conventional irrigant in endodontics, which, despite its broad acceptance, is associated with avoidable adverse effects. Moreover, the study seeks to ascertain whether the antimicrobial efficacy of these irrigants against *Enterococcus faecalis* (*E. faecalis*) is amplified when subjected to laser activation. Sixty single-rooted human teeth, decoronated at the Cemento-enamel Junction (CEJ), underwent biomechanical preparation. *E. faecalis* was introduced to form a biofilm over 21 days. The root samples were then divided into three experimental groups, each split into two subgroups: Group 1a - NaOCl, Group 1b - NaOCl and laser activation, Group 2a - triphala, Group 2b - triphala and laser activation, Group 3a - garlic extract, and Group 3b - garlic extract and laser activation. Microbial counts were assessed both pre- and post-irrigation to evaluate and compare the antimicrobial efficacy of the irrigants against *E. faecalis*. ANOVA with Tukey Post Hoc test and unpaired t-test (for parametric data) or Mann Whitney 'U' test (for non-parametric data). Highest mean reduction in bacterial colonies was recorded in laser activated NaOCl group while the lowest mean zone of inhibition was found in garlic extract without laser activation. Based on the findings of the present study, it can be concluded that laser activation substantially enhances the antimicrobial efficacy of herbal irrigants compared to non-activated NaOCl, demonstrating promising potential.

**Keywords:** Antimicrobial, Enterococcus Faecalis, Garlic extract, Laser activation, Sodium Hypochlorite, Triphala

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**Corresponding Author:** Treesa Mathew, Department of Conservative Dentistry and Endodontics, SMBT Dental College, Sangamner, Maharashtra, India

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#### INTRODUCTION

Most patients come to the dental clinic due to varying degrees of pain, primarily stemming from endodontic and periodontal issues. Identifying the endodontic source is crucial for clinicians to distinguish between odontogenic and non-odontogenic pain.<sup>[1]</sup>

The effectiveness of endodontic treatment is closely linked to managing infections. The current body of literature indicates that regardless of whether rotary, hand, or hybrid instrumentation is applied correctly, it may not entirely eliminate all organic and inorganic debris from the root canal system.

Due to this limitation, among others, irrigating solutions become crucial, compensating for the inadequacies of cleaning and enhancing overall endodontic decontamination procedures.<sup>[2]</sup>

The conventional irrigant for root canal cleansing and disinfection is sodium hypochlorite (NaOCl) solution, known for its antimicrobial and histolytic properties,

among others. However, the use of high concentrations of NaOCl solutions, particularly in single-visit treatment, raises concerns about cytotoxicity to periradicular tissues. The risk of irrigant extravasation into these tissues amplifies the worry of postoperative pain in such cases.<sup>[2]</sup>

For numerous years, garlic (*Allium sativum*) has served both as a culinary ingredient and a medicinal remedy, renowned for its anti-infective properties. Extensive experimental and clinical research has demonstrated that garlic extract possesses a range of beneficial effects, including antibacterial, antifungal, and antiviral properties, and it also reduces inflammation and modulates immunity. The antibacterial and antifungal attributes of garlic are primarily attributed to allicin (*diallyl thiosulfonate*), a compound with the capacity to impede bacterial cell growth.<sup>[3]</sup>

Triphala, an Ayurvedic formulation with historical and contemporary use in India, consists of three plants: *Phyllanthus emblica L.*, *Terminalia bellirica*, and *Terminalia chebula Retz.* Triphala is traditionally used to treat microbial infections, including wounds, ulcers, and dental cavities. Several studies have examined its effectiveness as an antibacterial agent and as a wound healer.<sup>[4]</sup>

Diode lasers are gaining attention as a supplementary tool for root canal disinfection. In the realm of laser technologies, the diode device stands out for researchers and clinicians due to its compact size, affordability, operating modes, and power outputs. Different wavelengths in endodontics for diode lasers have been investigated, including 810 nm, 830 nm, 940 nm, and 980 nm. Notably, the 940 nm wavelength has recently garnered interest, supported by evidence indicating the generation of a limited form of cavitation in aqueous fluids around the fibre tip's apex.<sup>[5]</sup>

Thus, through this study, we have evaluated the antimicrobial efficiency of herbal irrigants as alternatives to NaOCl and assessed the effect of laser activation on the efficacy of the irrigation solution.

#### MATERIAL AND METHODOLOGY

Sixty therapeutically extracted single-rooted permanent teeth with patent and mature root canals were selected. Immediately following collection, the teeth were immersed in a 3% NaOCl solution for an initial period of two hours. They were then meticulously cleaned of calculus, soft tissue remnants, attached bone, and other debris using ultrasonic scaling, after which they were sterilized by autoclaving. The teeth were subsequently stored in thymol until further use. After conducting a radiographic examination to confirm the presence of a single canal that is patent in each tooth, the teeth were consistently cut below the Cemento-enamel Junction (CEJ) using a diamond disk. This step is intended to establish a standardized 13 mm root length for each tooth included in the study.

Canal patency was confirmed using a 10K file, and subsequent instrumentation was carried out with the ProTaper Universal file system, extending up to file F3. Throughout the procedure, 2 mL of 3% NaOCl was applied between each instrument. Following this, irrigation with 17% ethylenediaminetetraacetic acid (EDTA) for a duration of 1 minute was performed to eliminate the smear layer. To prevent bacterial microleakage, the entire root surface of the tooth was coated with two layers of nail varnish. All samples were sterilized by autoclaving at 121°C and 15 psi for a duration of 15 minutes.

The American Type Culture Collection strain 29212 of *Enterococcus faecalis* (*E. faecalis*) was sourced from NFB Laboratories Private Limited located in Mumbai, India, from a stock culture stored at 4°C. Subsequently, it was incubated for 48 hours at 37°C in Mueller-Hinton agar. To generate a solution of *E.*

*faecalis*, the organism was transferred into Brain Heart Infusion (BHI) broth from a pure culture. The solution was kept at 37°C for 24 hours and then adjusted to a particular optical density using sterile BHI broth. Bacterial cells were resuspended in saline, resulting in a final concentration of approximately  $1.5 \times 10^8$  colony-forming units (CFU) per mL. The turbidity of the *E. faecalis* culture was adjusted to meet No. 0.5 McFarland standards.

Using a sterile micropipette, each root canal was fully filled with the infected broth. The samples were stationed in the test tubes and incubated at 37°C for 21 days. Throughout the span of incubation, fresh broth was added every 48 hours. Following the 21-day incubation period, saline irrigation was performed to remove the broth from the canals. Sterile paper points were used to collect samples from the root canal and cultured in Mueller-Hinton agar, with initial readings recorded for viable bacterial count. All procedures were conducted within a laminar airflow chamber. After collecting the samples, teeth were randomly divided into three main groups, which were subdivided into two each.

Group 1: (n=20)

1a (n=10)- Irrigation with NaOCl(5.25%) (5ml).

1b (n=10)- Irrigation with NaOCl(5.25%) with Laser Activation.

Group 2: (n=20)

2a (n=10)- Irrigation with Triphala (5ml). (Prepared by Jeevanrekha analytical services, Aurangabad, India)

2b (n=10)- Irrigation with Triphala with Laser Activation.

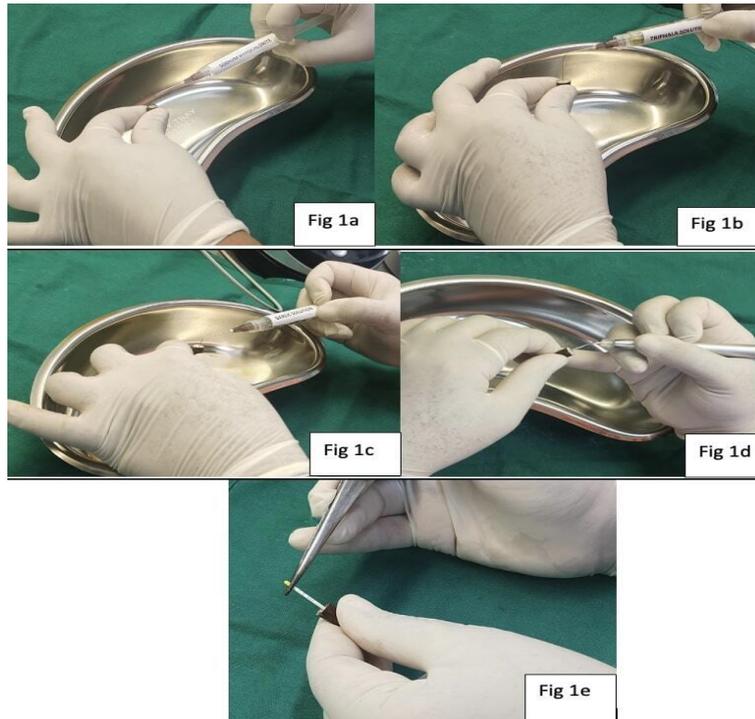
Group 3: (n=20)

3a (n=10)- Irrigation with *Allium Sativum* (5ml). (Prepared by Jeevanrekha analytical services, Aurangabad, India)

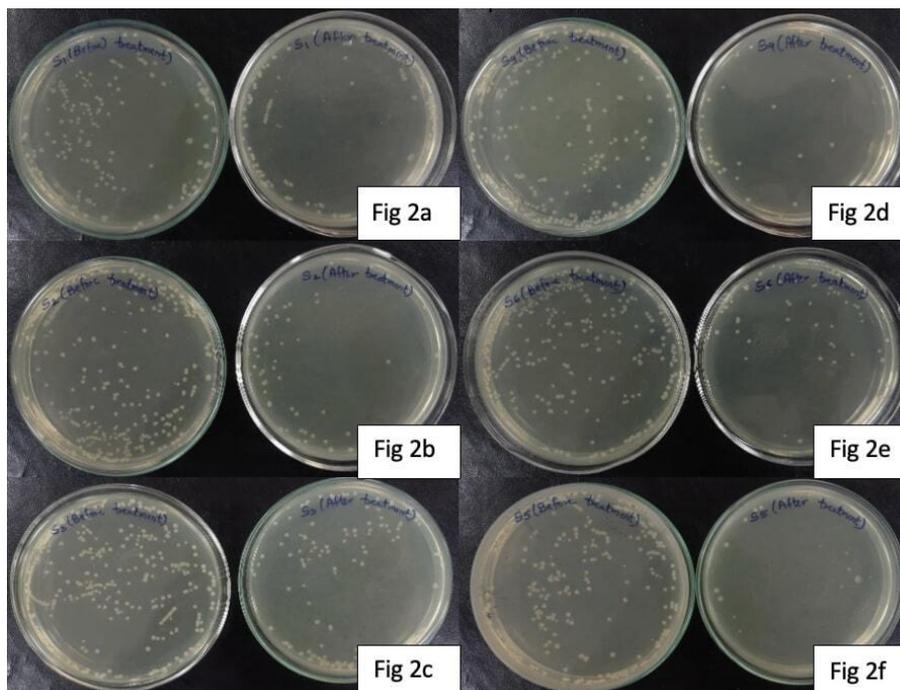
3b (n=10)- Irrigation with *Allium Sativum* with Laser Activation.

A diode laser (Biolase, Epic X, BIOLASE, Inc., USA) was used at a wavelength of 980 nm with a non-initiated tip at 1 watt in continuous motion. A lasing cycle comprised a 5-second activation of irradiation, succeeded by a 20-second halt, terminating 1 mm short of the apex. This lasing cycle was administered four times, utilizing 1.25 mL of the corresponding irrigation solution with each application. The total duration of radiation for each tooth amounted to 20 seconds.

Following this protocol, the irrigants were permitted to stay in the canals for a duration of 5 minutes. A final irrigation was subsequently executed utilizing 1 mL of sterile normal saline for each distinct sample. Samples were collected from the root canal system using sterile paper points. The paper points were carefully inserted into the canal to the working length for 10 seconds, before being transferred to tubes containing 5 mL of BHI broth. Subsequently, gathered samples underwent culture on Mueller-Hinton agar, and post-irrigation readings were recorded.



**Figure 1a- Irrigation with Sodium Hypochlorite (5.25%), 1b- Irrigation with Triphala, 1c- Irrigation with Garlic Extract, 1d- Laser activation, 1e- Collection of Sample with the help of Paper point**



**Figure 2a- CFU before and after activation with Sodium Hypochlorite, 2b- CFU before and after activation with Sodium Hypochlorite along with Laser Activation, 2c- CFU before and after activation with Triphala, 2d- CFU before and after activation with Triphala along with Laser Activation, 2e- CFU before and after activation with Garlic Extract, 2f- CFU before and after activation with Garlic Extract along with Laser Activation**

**RESULTS**

The percentage change in the CFU count of all six experimental groups is summarized in Table 1. A significant reduction is observed in all experimental

groups at baseline and postoperatively after irrigation ( $p < 0.001$ ).

The laser-activated NaOCl (Group 1b) exhibits significantly greater antimicrobial efficiency compared to Group 1a, which did not receive laser

activation. This pattern is consistent across other groups ( $p < 0.001$ ). NaOCl shows a significantly higher reduction in microbial count when compared to Triphala and garlic extract ( $p < 0.001$ ). Laser-

activated Triphala and garlic extract demonstrate superior results compared to non-laser-activated NaOCl ( $p < 0.001$ ) (see Table 2).

**Table 1: Overall Comparative statistics of Antimicrobial Efficacy Between Sodium Hypochlorite and Herbal Irrigants Like Triphala and Allium Sativum With and Without Laser Activation Against Enterococcus Faecalis respectively**

	Mean (Percentage Change)	SD	Minimum	Maximum	P value, Significance
Group 1A (NaOCl)	68.08	3.45	63.2	73.9	P<0.001**
Group 1B (NaOCl + Laser)	<b>82.46</b>	<b>1.78</b>	<b>80.22</b>	<b>85.71</b>	
Group 2A (Triphala)	<b>61.58</b>	<b>1.96</b>	<b>58.25</b>	<b>63.64</b>	
Group 2B (Triphala + Laser)	<b>77.55</b>	<b>3.46</b>	<b>71.17</b>	<b>82.31</b>	
Group 3A (Allium Sativum)	<b>59.90</b>	<b>2.92</b>	<b>55.43</b>	<b>63.43</b>	
Group 3B (Allium Sativum + Laser)	<b>73.35</b>	<b>1.99</b>	<b>69.95</b>	<b>76.22</b>	

\*\*p< 0.001 – highly significant difference

**Table 2: Pairwise comparative statistics of Antimicrobial Efficacy Between Sodium Hypochlorite and Herbal Irrigants Like Triphala and Allium Sativum With and Without Laser Activation Against Enterococcus Faecalis respectively**

Groups	Comparison Group	Mean Difference	p value Significance
<b>Group 1A (NaOCl) vs</b>	Group 1B (NaOCl+Laser)	<b>14.38</b>	<b>p&lt;0.001**</b>
	Group 2A (Triphala)	<b>6.49</b>	<b>p&lt;0.001**</b>
	Group 2B (Triphala+Laser)	<b>9.47</b>	<b>p&lt;0.001**</b>
	Group 3A (Allium Sativum)	<b>8.17</b>	<b>p&lt;0.001**</b>
	Group 3B (Allium Sativum+Laser)	<b>5.27</b>	<b>p=0.001*</b>
<b>Group 1B (NaOCl+Laser)</b>	Group 2A (Triphala)	<b>20.88</b>	<b>p&lt;0.001**</b>
	Group 2B (Triphala+Laser)	<b>4.91</b>	<b>p=0.002*</b>
	Group 3A (Allium Sativum)	<b>22.56</b>	<b>p&lt;0.001**</b>
	Group 3B (Allium Sativum+Laser)	<b>9.11</b>	<b>p&lt;0.001**</b>
<b>Group 2A (Triphala) vs</b>	Group 2B (Triphala+Laser)	<b>15.96</b>	<b>p&lt;0.001**</b>
	Group 3A (Allium Sativum)	<b>1.67</b>	<b>p=0.731</b>
	Group 3B (Allium Sativum+Laser)	<b>11.76</b>	<b>p&lt;0.001**</b>
<b>Group 2B (Triphala+Laser) vs</b>	<b>Group 3A (Allium Sativum)</b>	17.64	<b>p&lt;0.001**</b>
	<b>Group 3B (Allium Sativum + Laser)</b>	4.19	<b>P=0.012*</b>
<b>Group 3A (Allium Sativum) vs</b>	<b>Group 3B (Allium Sativum + Laser)</b>	13.44	<b>p&lt;0.001**</b>

p>0.05 – no significant difference

\*p<0.05 – significant

\*\*p<0.001 – highly significant

**DISCUSSION**

*E. faecalis* is a common bacterium associated with secondary infections and is one of the primary organisms responsible for persistent infections and eventual failure of root canal therapy. NaOCl is used as the gold standard root canal irrigant. Although effective, it can cause certain side effects that are avoidable. Herbal irrigants have gained popularity due to their natural properties and comparatively greater biocompatibility. The potential of lasers in endodontics has been under research and has shown promising results. Thus, this study compares the

antimicrobial efficacy of NaOCl with herbal irrigants—specifically Triphala and garlic extract—against *E. faecalis*, with and without laser activation. Group 1b (NaOCl + Laser) shows the highest percentage reduction in microbial count, followed by Group 2b (Triphala + Laser) and then Group 3b (Garlic Extract + Laser). These three subgroups are followed by Group 1a (NaOCl), then Group 2a (Triphala), with the least percentage reduction shown by Group 3a (Garlic Extract) (Table 1). The reduction in microbial count has been assessed, and the results indicate that in Group 1, the laser-

activated NaOCl (Group 1b) demonstrates significantly better antimicrobial efficiency compared to Group 1a, which did not receive laser activation. This result was consistent across all other groups as well, where the laser-activated groups (Group 2b and Group 3b) showed significantly greater reductions in microbial count compared to the non-laser-activated subgroups (Group 2a and Group 3a) (Table 2). Thus, it can be concluded that laser activation enhances the antimicrobial properties of the irrigation solution.

Groups 2b (Triphala + Laser) and 3b (Garlic Extract + Laser) show significantly better results compared to Group 1a, leading to the conclusion that laser-activated herbal irrigants exhibit superior antimicrobial properties compared to NaOCl without laser activation (Group 1a). Therefore, we deduce that laser activation has a significant impact on the efficacy of the irrigation solution (Table 2).

Group 1a (NaOCl) demonstrates a major reduction in microbial count compared to Group 2a (Triphala) and Group 3a (Garlic Extract), thus affirming the superiority of NaOCl over Triphala and garlic extract (Table 2).

The results of this study indicate that laser activation significantly affects the reduction of *E. faecalis* counts, irrespective of the irrigant used. While NaOCl shows the best results when activated with laser (Group 1b) (Table 1), herbal irrigants can also be used with laser activation to reduce bacterial counts. When comparing non-laser-activated NaOCl (Group 1a) with activated herbal irrigants (Groups 2b and 3b), the antimicrobial efficiency of the latter is found to be significantly better (Table 2). Given the disadvantages of NaOCl when in contact with soft tissue, a viable herbal alternative is in great demand. Thus, clinical trials are necessary to ascertain the effectiveness of herbal irrigants along with laser activation when used in vivo.

Vinod V. Panchal *et al.* conducted an in vitro study, concluding that while Triphala exhibited notably superior antibacterial activity, it was still less effective compared to NaOCl.<sup>[6]</sup> Ourvind J. S. Birring *et al.* also conducted an in vitro study comparing garlic extract and NaOCl, concluding that garlic extract may provide benefits as an herbal root canal irrigant in preventing *E. faecalis* biofilm formation inside root canals and may effectively penetrate into root dentin. However, the lack of sufficient in vivo studies prohibits its clinical recommendation at present.<sup>[7]</sup> In an ex vivo study by Ekta Choudhary *et al.*, it was found that Triphala displayed superior antibacterial efficacy against *E. faecalis*, whereas the antifungal effect against *Candida albicans* was more pronounced with *Morindacitrifolia* juice (MCJ). Nevertheless, both herbal irrigants demonstrated statistically comparable overall antimicrobial efficacy against the two tested microorganisms.<sup>[8]</sup>

Laser-activated irrigation using the Photon-induced photo acoustic streaming (PIPS) protocol and NaOCl significantly enhanced antimicrobial effects by

eliminating bacterial biofilm in vitro, suggesting that PIPS is a promising adjunctive method to conventional root canal therapy, as concluded by an in vitro study conducted by Mohammed Al Shahrani *et al.*<sup>[9]</sup> In another study by Shanshan Dai *et al.*, it was found that when a diode laser is used, the combination of NaOCl and laser activation was more effective than laser activation alone for disinfecting infected root canals of primary teeth.<sup>[10]</sup> An in vivo study by Namrata Mehta *et al.* concluded that a power setting of 1.5 W using a 940 nm diode laser produced significant results in reducing both anaerobic and aerobic bacterial growth.<sup>[11]</sup> In contrast, ultrasonically activated chitosan demonstrated a lower viable bacterial count compared to diode laser-activated chitosan, attributed to the broader movement and contact of the irrigant across a larger surface area, as observed in an in vitro study conducted by Krishna Prasada *et al.*<sup>[12]</sup> The greatest reduction in bacterial load was observed in the group utilizing the highest power setting of a diode laser in combination with NaOCl, as compared to other power outputs. This finding was reported in an in vivo study by Mahima Tilakchand *et al.*<sup>[13]</sup> The output power of a diode laser also plays a pivotal role, as highlighted in the aforementioned study. An in vivo study by Snehal Sonarkar *et al.* concluded that for aerobic bacteria, photo-activated disinfection (PAD) proved to be more effective than 5% NaOCl, while the diode laser was the least effective. For anaerobic bacteria, both PAD and 5% NaOCl demonstrated superior antibacterial activity compared to the diode laser.<sup>[14]</sup>

Thus, the combination of an irrigant with laser activation improves the effectiveness of the irrigation protocol. The properties of the irrigant also play an important role in determining the effectiveness of the protocol. Various studies have been conducted with different combinations of herbal irrigants, but the effect of laser activation on these irrigants needs further exploration. Clinical trials are necessary to determine the effect of the irrigation protocol when performed in vivo.

## CONCLUSION

NaOCl continues to set the gold standard when activated with laser, demonstrating significantly higher antimicrobial efficiency compared to other experimental groups. Herbal irrigants also show significant antimicrobial efficiency when activated with laser, in comparison to NaOCl without laser activation. Thus, herbal irrigants with laser activation have shown promising results in vitro, according to this study. Further in vivo studies should be conducted to validate the findings of this study.

**Limitations:** The conclusion of this study requires further assessment to confirm its clinical relevance.

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