

# Clinical Quantitative Antibacterial Potency of Garlic-Lemon Against Sodium Hypochlorite in Infected Root Canals: A Double-blinded, Randomized, Controlled Clinical Trial

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**ABSTRACT** **Introduction:** Sodium hypochlorite for endodontic treatment has been shown to exhibit significant antimicrobial properties, with adverse effects such as sodium hypochlorite accidents. Natural irrigants have shown significant antimicrobial action and the added advantage of being biocompatible. This study proposes an alternative intracanal irrigant made from Garlic-Lemon (Ga-Li) extract. **Aim:** To evaluate the antimicrobial action of 1.8% Garlic-Lemon (Ga-Li) mixture in contrast to 3% sodium hypochlorite in a tooth diagnosed with asymptomatic apical periodontitis. **Materials and Methods:** Thirty patients were randomly allocated into two groups: Group A, 3% sodium hypochlorite and Group B, 1.8% Garlic-Lemon. Single- or multirrooted teeth root canals were instrumented and prepared by using ProTaper Gold. Root canal samples were taken both pre- and postinstrumentation. These samples were subjected to DNA extraction, amplification, and quantification by using a real-time polymerase chain reaction (qPCR). **Results:** Samples before preparation (S1) were tested positive for microbial presence, with mean numbers of  $7.0 \times 10^7$  and  $12.4 \times 10^7$  bacterial cells for the sodium hypochlorite and Garlic-Lemon groups, respectively. Postpreparation (S2), in sodium hypochlorite and Garlic-Lemon groups, bacterial counts were still present with mean counts seen at  $27.4 \times 10^5$  and  $7.7 \times 10^5$  bacterial cells, respectively. Intergroup comparison resulted in a statistically insignificant difference ( $P > 0.05$ ). **Conclusion:** Garlic-Lemon has shown microbial load reduction that is as effective as sodium hypochlorite, with the highest mean bacterial reduction percentage. The results of the present randomized, controlled clinical trial suggest that Garlic-Lemon is a potential new alternative as an endodontic irrigant.

**KEYWORDS:** Apical periodontitis, bacteria, disinfection, irrigant, microbial reduction, molecular biology, polymerase chain reaction

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## INTRODUCTION

The aim of endodontic treatment in teeth with infected root canals with apical periodontitis is to promote optimal reduction of bacterial load.<sup>[1]</sup> Lipopolysaccharides (LPS) are the most important by-products present on the outer layer of gram-negative bacterial species. Root canal infections are

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polymicrobial, where the most frequently noted bacteria in teeth diagnosed with asymptomatic apical periodontitis include *Porphyromonas endodontalis*, *Dialister invisus*, *Olsenella uli*, and *Fusobacterium nucleatum*.<sup>[2]</sup> Mechanical instrumentation for these case scenarios is shown to be insufficient to remove bacteria due to the complexity of root canal morphology. Therefore, cleaning of the root canal system plays a crucial role in the success of endodontic treatment, which is achieved by using various chemo-mechanical preparation and irrigation methods.

According to Zehnder, an ideal root canal irrigant should exhibit adequate antimicrobial action, be nontoxic to the body, exert the least cytotoxic effect on tissues with the ability to dissolve necrotic pulp tissue, as well as inactivate endotoxins while also facilitating smear layer removal.<sup>[3]</sup> Many irrigants have been investigated till date by various authors, with none having to fulfill all the criteria mentioned earlier.

Sodium hypochlorite (NaOCl) is widely used in endodontics as an irrigating solution due to its antimicrobial action as well as tissue-dissolving property.<sup>[4]</sup> However, the adjuvant use of secondary irrigating solutions is deemed essential for effective removal of the smear layer since NaOCl is shown to be effective only against organic tissues and microbes.<sup>[5]</sup> The drawbacks of the usage of sodium hypochlorite are numerous, such as unpleasant taste, cytotoxicity, and sodium hypochlorite accident. A sodium hypochlorite accident occurs when it extrudes beyond the apex or by an accidental injection that is shown to exhibit adverse reactions such as tissue necrosis. However, the incidence of this occurrence can be reduced by using side-vented needles, passively fitting the needle in the root canal and injecting the irrigants at a gentle flow rate.

In an *in vitro* study, it was found that both fresh and ethanolic extracts of Garlic-Lemon exerted better antimicrobial efficacy in comparison to sodium hypochlorite.<sup>[6]</sup> The presence of citric acid has shown a remarkable effect in aiding in the penetration of irrigants into dentinal tubules and, in turn, providing optimal disinfection of the complex canal anatomy.

This study aims at conducting a Randomized Clinical Trial (RCT) to evaluate the antimicrobial action of both Garlic-Lemon (Ga-Li) and sodium hypochlorite (NaOCl) in teeth diagnosed with asymptomatic apical periodontitis.

## MATERIALS AND METHODS

This study followed the revised guidelines of the Consolidated Standards of Reporting Trials Statement.<sup>[7]</sup>

## STUDY DESIGN

The present clinical trial was carried out after obtaining approval from the Institutional Scientific Review Board of the University with an ethical clearance number (SDC/MDS/18–19/0123). It was registered in the Clinical Trials Registry of India (CTRI) with approval number CTRI/2020/08/027260. Thirty patients who had visited the postgraduate clinic were randomly enrolled to the study groups after assessing the criteria for inclusion. No dropouts were found. The various phases of sample collection were conducted by a single operator throughout the study timeframe.

## FORMULATION OF SAMPLE SIZE

Sample size was formulated based on the results achieved by the pilot study. The sample size was calculated as 7 in each group, with an allocation ratio set at 1:1. G\*Power 3.1.9.4 was the software analysis used for software determination, with an alpha error at 5% and a statistical power of 95%.

## SAMPLE CHARACTERISTICS

Samples from the root canal were taken from 30 participants (12 males and 18 females, with age ranging from 18 to 75 years with a mean age of 37 years).

## RANDOMIZATION

The evaluators and patients of this study were subjugated to a double-blinded technique. Randomization was done earlier by a third operator with a predetermined allocated sample ( $n = 30$ ). The randomization process was carried out by using block randomization and the method of the table of random numbers. For allocation concealment, sequentially numbered, opaque, sealed envelope (SNOSE) method was executed where a allocation group mentioned papers were sealed in a dark-colored envelope containing serial numbers was prepared by the third operator. The treatment protocol assigned for respective groups was sealed as well in their respective envelopes. Study numbers were assigned to patients based in sequence. The envelope was subjected to opening once the groups were assigned. On the basis of the group assigned, the respective treatment was carried out by the first operator.

## CASE SELECTION

A total of 30 patients with an indication for root canal treatment were enrolled in this study. Inclusion criteria for these participants were as follows: asymptomatic apical periodontitis, single- and multirrooted teeth, pulp chamber walls intact, teeth diagnosed with necrotic pulp, and apical periodontitis confirmed with digital radiograph and pulp sensibility testing. In case of multirrooted teeth, palatal and distal canals of maxillary and mandibular molars were only included. Exclusion

criteria were as follows: teeth subjected to gross carious lesion, fracture of the teeth, teeth requiring endodontic retreatment, and teeth with a periodontal pocket greater than 4mm.

#### PREPARATION OF GARLIC-LEMON

Fresh Garlic (*Allium sativum*) was purchased from the local market and was washed thoroughly by using distilled water. The dried garlic bulbs were ground by using an electronic miller. Then, 60g of garlic was placed in a tared extraction thimble and extracted with 95% ethanol in a Soxhlet extraction apparatus for about 3 hours. An equal amount of freshly prepared sterile Lemon (*Citrus Limon*) juice was added to make a final concentration, which was found to be 1.8%.

Informed consent was obtained from patients in either the English language or a regional language based on their familiarity and preference before initiating the treatment. The patients who fulfilled all the criteria for inclusion were subjugated to the following groups:

- Group A: 3% sodium hypochlorite (VENSONS INDIA)
- Group B: 1.8% Garlic-Lemon

#### SAMPLE COLLECTION

The sample collection from the root canals was conducted under strict aseptic conditions. Before sample collection, patients were given oral prophylaxis (0.12% chlorhexidine followed by removal of supragingival biofilm by scaling) before initiating treatment. The tooth of interest was isolated under rubber dam isolation, followed by which the operative field, including tooth, clamp, and the surrounding dam, was cleaned by using 3% hydrogen peroxide and 3% NaOCl. Sterile burs were used to complete access cavity preparation with the help of saline irrigation. The same disinfection protocol as described earlier was repeated, followed by which inactivation of the residue sodium hypochlorite was conducted by application of 5% sodium thiosulfate. Sterility control samples were collected by scrubbing sterile paper points against the cavosurface angle of the access cavity of the prepared tooth. For the tooth to be included in sterility control samples; the samples had to report negative for bacteria in an end point polymerase chain reaction (PCR) with the help of universal primers.

Two samples were taken from the participant: one preinstrumentation (S1) and one immediately postinstrumentation (S2). For samples taken before instrumentation, sodium thiosulfate solution was filled in the pulp chamber without overflowing, and a 10 No K-File was used to carry the solution into the canal; in

addition to this, the canal walls were gently filed in a watch winding motion to suspend the canal contents in the solution. Consecutively, sterile paper points were placed in the canal to a level of approximately 1mm short of the predetermined apex length via a digital radiograph without having any contact to access cavity walls. The samples were later collected in a 1.5ml sterile DNase/RNase-free tube containing 10% Sodium Dodecyl Sulfate (Cat#194831, MP Bio, Canada) and 10% Triton X100 (Cat#64518, SRL Chemicals, India). The study groups were instrumented by using ProTaper Gold Rotary File System (Dentsply Maillefer, Ballaigues, Switzerland). The total volume of irrigation during the procedure for both Garlic-Lemon and NaOCl groups was maximum at 15ml. Final irrigation with saline was conducted, followed by a final rinse of 5% sodium thiosulfate being done to inactivate the chlorination effect of sodium hypochlorite. The postpreparation (S2) samples were collected by using a paper point from two groups. Samples taken during the procedure were stored in 4C until they were transported to the laboratory for DNA extraction.

#### REAL-TIME POLYMERASE CHAIN REACTION

DNA extraction, amplification, quantitation of the total amount of bacteria, and establishment of standards were as described based on previous studies.<sup>[8-10]</sup> The samples were then examined by a real-time polymerase chain reaction in the presence of QuantiNova SYBR Green PCR Kit (Cat#208052, Qiagen, Germany) in Qiagen 5-plex rotor gene real-time polymerase chain reaction system to demonstrate a linear standard curve graph.

#### STATISTICAL ANALYSIS

A normality test was conducted (Kolmogorov–Smirnov and Shapiro–Wilks tests), and it showed that the data did not follow a normal distribution. Nonparametric tests were done to analyze the data. To assess the data, SPSS software (IBM Statistics, Ver 23.0, Armonk, NY, IBM Corp) was used. The significance level for all tests was set at  $\alpha = 0.05$ , with a p value  $< 0.05$  being considered statistically significant. For a comparison of various intergroup intervals (S1, S2, and fold difference), Kruskal–Wallis test was used followed by Bonferroni-adjusted Mann–Whitney test for multiple pairwise comparisons. The null hypothesis is that Garlic-Lemon was more antimicrobial than sodium hypochlorite.

#### RESULTS

None of the sterility control samples were found to be positive for bacteria; hence, none were excluded for the assessment. The process of treatment protocol

is explained in Figure 1 according to CONSORT guidelines. The tested S1 samples from all the participants were confirmed with positive bacterial presence by qPCR using the 16S rRNA-based primer. Comparison among these samples (S1) was found to be statistically insignificant ( $P > 0.05$ ). [Table 1] denoted the quantitative real-time polymerase chain reaction data.

A mean number of  $7.0 \times 10^7$  bacterial cells occurred in samples of S1 for the sodium hypochlorite group. Total bacterial counts in S2 were substantially reduced to a mean of  $27.4 \times 10^5$  cells. It was found that the mean fold difference was  $5.47 \times 10^2$ . In the Garlic-Lemon group, the mean number in S1 samples was seen as  $12.4 \times 10^7$  cells, which was found to be reduced in S2 samples to the mean number of  $7.7 \times 10^5$  bacterial cells. The fold difference of samples was found to exhibit a mean of  $13.23 \times 10^2$  bacterial cells. No statistically significant difference between groups was revealed by S2 ( $P > 0.05$ ). However, when comparing the fold difference, there was no statistically significant difference between sodium hypochlorite and Garlic-Lemon ( $P > 0.05$ ). [Table 2] summarizes the Box plot values, including the median, first quartile, and third quartile. The Box plot comparing groups for S1, S2, and fold difference is shown in Figures 2–4.

## DISCUSSION

This study introduces an alternative intracanal irrigant for conventional endodontic procedures. The aim of the current randomized, controlled clinical trial is to compare the effectiveness of sodium hypochlorite and Garlic-Lemon irrigant in root canals of teeth diagnosed with asymptomatic apical periodontitis. The null hypothesis was simply dismissed, because there was no substantial difference between groups in microbial reduction, as Garlic-Lemon was as effective as sodium hypochlorite.

Endodontic irrigating solutions are mandatory along with mechanical instrumentation for the thorough cleaning of the root canal system.<sup>[11]</sup> The precipitate formation and presence of para-chloroaniline (PCA) is a major drawback when using a different combination of chemical irrigants, for instance sodium hypochlorite (NaOCl) and chlorhexidine (CHX).<sup>[12]</sup> Recently, the International Agency for Research on Cancer (IARC) has shown that endodontic irrigating solutions such as sodium hypochlorite (NaOCl), chlorhexidine (CHX), and ethylenediaminetetraacetic acid (EDTA) had carcinogens and were classified mainly as Group 1 carcinogens. However, there was no presence of toxic metals in alternative irrigants such as herbal irrigants, consisting of garlic, neem, tulsi, and *A. vera*.<sup>[13]</sup> Herbal irrigants have also shown potential to detoxify heavy

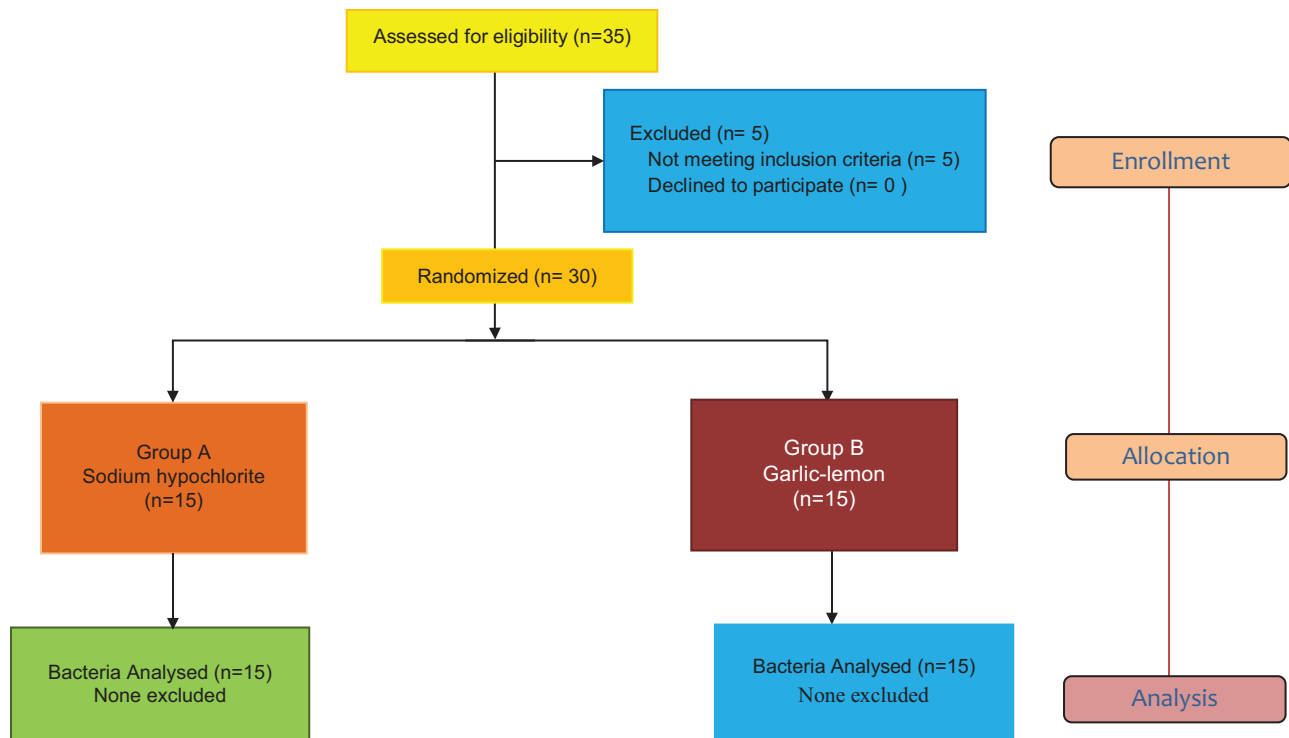


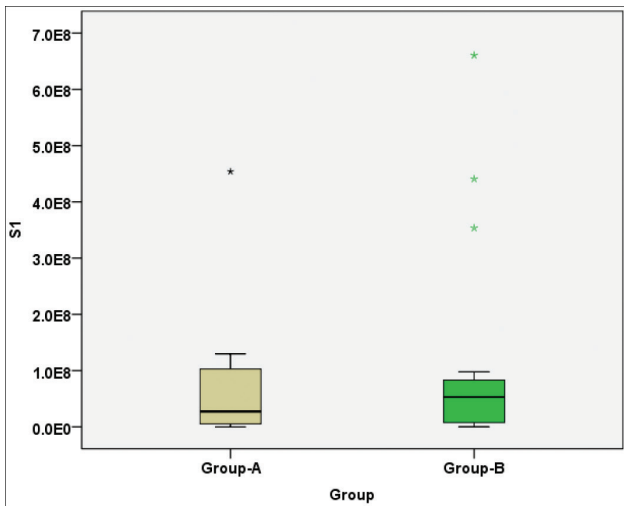
Figure 1: Consolidated standards of reporting trials flowchart

**Table 1: Total bacterial load in root canal samples of teeth with asymptomatic apical periodontitis taken before (S1) and after instrumentation using various irrigants such as sodium hypochlorite and garlic-lemon**

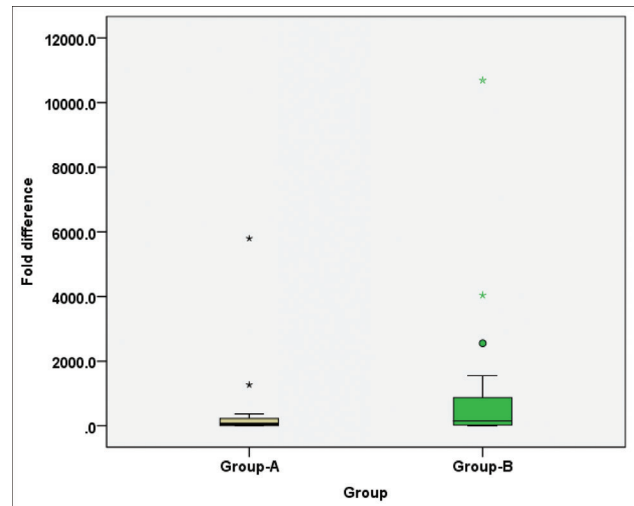
Instrumentation groups	Mean (copies/ $\mu$ l)			p-value	Mean % S1 to S2 reduction
	S1	S2	Fold difference (S1/S2)		
Sodium hypochlorite	$7.0 \times 10^7$	$27.4 \times 10^5$	$5.47 \times 10^2$	0.191	96.10
Garlic-lemon	$12.4 \times 10^7$	$7.7 \times 10^5$	$13.23 \times 10^2$		99.37

**Table 2: Box plot values showing median, first quartile, and third quartile**

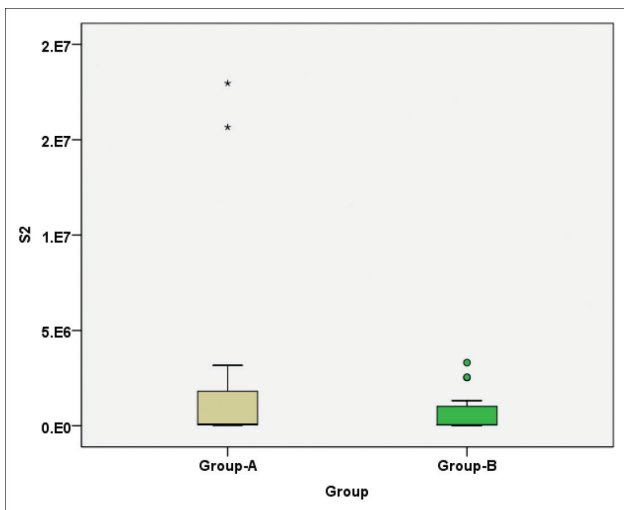
Instrumentation groups	Median			First quartile			Third quartile		
	S1	S2	Fold difference (S1/S2)	S1	S2	Fold difference (S1/S2)	S1	S2	Fold difference (S1/S2)
Sodium hypochlorite	$2.73 \times 10^7$	$7.02 \times 10^4$	$0.53 \times 10^2$	$8.97 \times 10^5$	$4.38 \times 10^4$	$0.2 \times 10^1$	$1.16 \times 10^8$	$2.20 \times 10^6$	$2.77 \times 10^2$
Garlic-lemon	$5.29 \times 10^7$	$4.73 \times 10^4$	$1.50 \times 10^2$	$71.12 \times 10^5$	$3.11 \times 10^4$	$2.1 \times 10^1$	$0.97 \times 10^8$	$1.32 \times 10^6$	$15.47 \times 10^2$



**Figure 2: S1 samples - box plot demonstrating the bacterial load of samples taken before instrumentation (S1 samples) in each group**



**Figure 4: Fold difference - box plot demonstrating the fold difference (S1/S2) in each group**



**Figure 3: S2 samples - box plot demonstrating the bacterial load of samples taken after instrumentation (S2 samples) in each group**

metal-induced damage. Therefore, it is important to standardize irrigation protocol and to promote the use of herbal irrigants as a final irrigant to detoxify heavy metal-induced damage.

The progressive research in this field has enabled researchers to emphasize more on herbal irrigants, which are shown to exert beneficial physiologic effects and curative properties such as anti-inflammatory action, antioxidant action, and radical scavenging activity. Garlic (*Allium sativum*) shows a wide range of therapeutic effects due to the presence of allicin and other thiosulfonates. Han *et al.* have shown that 1 mg of allicin has a similar effect as that of 15IU of penicillin. Various studies have reported that garlic inhibited the growth of multiple microorganisms that were classified as gram positive and gram negative.<sup>[14,15]</sup> A recent study by Rao *et al.* showed garlic to have significant

tissue-dissolving capacity when checked under different concentrations at different time intervals under laboratory conditions.<sup>[16]</sup> One major disadvantage of garlic is its pungent odor. This disadvantage can be overcome by the use of lemon.

Lemon (*Citrus Limon*) has been shown to help in reducing the pungent smell of garlic by impeding the production of volatile sulfur compounds.<sup>[17]</sup> Lemon has been shown to have a pH of 2.21, with additional curative properties such as antibacterial, antiviral, antifungal, anti-inflammatory, and anticancerous activities.<sup>[18,19]</sup> The components of lemon juice include ascorbic acid, phenolic acids, polyphenols, and dietary fibers. Different studies have demonstrated lemon's antimicrobial effectiveness against a wide variety of bacteria.<sup>[20,21]</sup> Lemon solution has also shown additional properties, making it significant for endodontic procedures such as removal of the smear layer present on the root canal dentin walls.<sup>[22]</sup>

Both garlic and lemon solutions are biologically compatible materials, as they are frequently used during meals. Garlic is known to possess antioxidant properties, which have shown protective properties against blood vessels from oxidative stress induced by  $H_2O_2$ .<sup>[23]</sup> Another factor seen is the inhibition of helper T cells and inflammatory cytokines.<sup>[24]</sup> Garlic, on the other hand, is shown to possess structural components such as allicin and ajoene, which have the potential to act as regulators of immunity with combined anti-inflammatory action, inhibiting the synthesis of nitric oxide in macrophage-like cells. Macrophages are known to produce proinflammatory mediators such as nitrogen oxide, which are shown to inhibit cyclooxygenase and 5-lipoxygenase enzymes and, in turn, inhibit inflammation cytokines and prostaglandins.<sup>[25]</sup> Lemon extract is known to be a rich natural reservoir of Vitamin C, and it shows powerful antioxidant properties. The anti-inflammatory activities of flavonoids present in lemon are as follows: Antioxidant and radical scavenging activities, control of cellular function of inflammation cells, modulation of the function of arachidonic acid metabolism enzymes (phospholipase A2, cyclooxygenase lipoxygenase) and nitric oxide synthase, modulation of the development of other proinflammatory molecules, and modulation of proinflammatory gene inevitably lead to a reduction in the levels of ESR and serum CRP.<sup>[26]</sup> In a study by Galati *et al.* (2015), it has been proven that lemon mucilage has been shown to substantially inhibit carrageenan-induced edema in the rat paw from 59% to 73.5%.<sup>[27]</sup> Given the current evidence of scientific literature, it can be hypothesized that the mechanism of action of

the Garlic-Lemon extract could have the properties mentioned earlier in an additive beneficial way, though more research needs to be focused on proving its interaction properties and its effects in the long run. To sum it up, the combination of Garlic-Lemon may have properties of tissue dissolving, smear layer removal, antibacterial, anti-inflammatory, antifungal, antiviral, anticarcinogenic, and antioxidants. Further research could prove beneficial to improve the efficacy of this combination.

Intragroup quantitative analysis revealed that both sodium hypochlorite and Garlic-Lemon were found to be highly successful in reducing the levels of bacteria inside the root canal. The mean sodium hypochlorite fold difference was noted to be  $5.47 \times 10^2$  (median,  $0.53 \times 10^2$ ), whereas the Garlic-Lemon group demonstrated  $13.23 \times 10^2$  (median,  $1.50 \times 10^2$ ). This was in agreement with the research done by Rocas and Siqueira (2011), who emphasized that reducing microbial load within the root canal to a controllable level plays a vital role with the help of chemo-mechanical debridement.<sup>[28]</sup> The mean percentage reduction from S1 to S2 for both sodium hypochlorite and Garlic-Lemon was found to be 96.10% and 99.37%, respectively. The difference in the percentage reduction of microbial load clearly shows that Garlic-Lemon can be used in endodontic treatment as an alternative irrigating agent. More research can be focused on this combination to make use of its beneficial effects as various adjuvants such as intracanal medicaments and even in regenerative endodontics, as chances of its toxicity levels are significantly minimal.

Root canal morphology is shown to be profusely complex, making it difficult for complete disinfection as pulp tissue and microbial remnants may persist in the irregularities of canal. According to Wu and Wesselink, 65% of uninstrumented areas were reported in instrumented oval canals.<sup>[29]</sup> This indicates the need for proper disinfection of the apical third. Due to reports showing various accidents on the usage of sodium hypochlorite, clinicians show extreme concern while irrigating with this solution, especially in the lower third of the root canal system. Herbal irrigants can prove a significant role as an alternative since they are less caustic to the tissues. In this study, Garlic-Lemon has been shown to exhibit maximum disinfection with the highest mean bacterial reduction percentage, showing that it can successfully fulfill the apical region disinfection of the root canal with minimal complications even on apical extrusion.

The overall percentage reduction of bacteria from S1 to S2 for Garlic-Lemon was better than previous

studies, where 2.5% sodium hypochlorite was used as a standard irrigant (95.9%, 96.9%, and 95.2% mean).<sup>[30,31]</sup> In another recent study by Siddique *et al.* (2020),<sup>[10]</sup> it was seen that mechanical instrumentation with ProTaper Gold with 3% NaOCl showed 95.72% (matches with results of our study, i.e., ProTaper Gold with 3% NaOCl showed 96.10% bacterial load reduction) bacterial reduction; however, in the current study, the same instrumentation in combination with Garlic-Lemon showed 99.37% bacterial load reduction.

The major limitation of the current study was the technique of sample collection using paper points, which was restricted to a single portion of the root canal system, thereby limiting the data on bacterial counts in the primary canals only and not taking into account the accessory canals.

## CONCLUSIONS

Garlic-Lemon has shown promising results and is, hence, considered an alternative root canal irrigant in comparison with sodium hypochlorite. The antimicrobial efficacy of Garlic-Lemon was found to be as effective as sodium hypochlorite, with a higher mean bacterial reduction percentage. Further studies should be carried out to analyze the substantivity of this combination with other chemical irrigants.

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Nil.

## CONFLICTS OF INTEREST

There are no conflicts of interest.

## AUTHORS' CONTRIBUTIONS

Riluwan Siddique - concepts, design, definition of intellectual content, literature search, clinical studies, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing and review. Manish Ranjan - manuscript editing and review. Jerry Jose - manuscript editing and review. Ankita Srivastav - manuscript editing and review. R. Rajakeerthi - manuscript editing and review. Ajith Kamath - manuscript editing and review.

## ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

The present clinical trial was carried out after obtaining approval from the Institutional Scientific Review Board

of the University with an ethical clearance number SDC/MDS/18–19/0123.

## PATIENT DECLARATION OF CONSENT

Informed written consent was obtained from all the participants for participation in the study, publication of the data for research, and educational purposes.

## DATA AVAILABILITY STATEMENT

The data set used in this study is available from corresponding author (Dr. Riluwan Siddique, e-mail: riluwanas@gmail.com) on request.

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