ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at:www.ijdsir.com

Volume – 9, Issue – 5, October – 2026, Page No. : 156 – 163

Evaluation of Antimicrobial Efficacy of 5.25% Sodium Hypochlorite Versus 100% Garlic Oil Against Porphyromonas Gingivalis on Contaminated Endodontic K-files: An in Vitro Study

[1]Dr. Sanjana K Hiremath, Postgraduate Student, Department of Pediatric and Preventive Dentistry, SDM College of Dental Sciences and Hospital, A Constituent Unit of Shri Dharmasthala Manjunatheshwara University, Dharwad, Karnataka, India

[2]Dr. Shruthi B Patil, Professor, Department of Pediatric and Preventive Dentistry, SDM College of Dental Sciences and Hospital, A Constituent Unit of Shri Dharmasthala Manjunatheshwara University, Dharwad, Karnataka, India

Corresponding Author: Dr. Sanjana K Hiremath, Postgraduate Student, Department of Pediatric and Preventive Dentistry, SDM College of Dental Sciences and Hospital, A Constituent Unit of Shri Dharmasthala Manjunatheshwara University, Dharwad, Karnataka, India

Citation of this Article: Dr. Sanjana K Hiremath, Dr. Shruthi B Patil, "Evaluation of Antimicrobial Efficacy of 5.25% Sodium Hypochlorite Versus 100% Garlic Oil Against Porphyromonas Gingivalis on Contaminated Endodontic K-files: An in Vitro Study", IJDSIR – October – 2026, Volume – 9, Issue – 4, P. No. 156 – 163.

Copyright: ©2026 Dr. Sanjana K Hiremath, et al. This is an open access journal and article distributed under the terms of the creative common's attribution non-commercial License. Which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given, and the new creations are licensed under the identical terms.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Aim & Background: This in vitro study aimed to compare the antimicrobial efficacy of 5.25% sodium hypochlorite (NaOCl) versus 100% garlic oil against Porphyromonas gingivalis on contaminated endodontic K-files. Porphyromonas gingivalis is a key pathogen in endodontic–periodontal lesions. While 5.25% NaOCl is the gold standard disinfectant, its cytotoxicity on periapical tissues is a concern. Garlic oil, rich in allicin, offers a natural biocompatible alternative.

Methods: Forty sterilized #10 no. K-files were contaminated with P. gingivalis and divided into four groups based on disinfectant and exposure time of 5 and 15 minutes, then files were inoculated on blood agar and incubated anaerobically at 37°C for 48 hours. Colony-forming units (CFUs) were counted and analyzed statistically ($p < 0.05$).

Results: A statistically significant difference in antimicrobial efficacy was observed between the two agents at both time intervals ($p < 0.001$), with garlic oil demonstrating promising antimicrobial activity, suggesting its potential as a biocompatible adjunct.

Conclusion: While 5.25% NaOCl remains the conventional irrigant of choice, 100% garlic oil demonstrates appreciable antimicrobial activity, suggesting its potential as a biocompatible natural adjunct in endodontic disinfection.

Clinical Significance: This study suggests 100% garlic oil as a promising natural alternative or adjunct for disinfecting endodontic K-files, offering better biocompatibility and lower cytotoxicity, especially beneficial in pediatric patients to minimize chemical exposure while supporting effective infection control.

Keywords: Sodium Hypochlorite, Porphyromonas Gingivalis, Garlic (Allium Sativum), Endodontics, Antimicrobial Activity

Introduction

Microbial infection is the primary etiological factor responsible for pulpal and periodontal diseases. The success of endodontic therapy largely depends on effective elimination of microorganisms from the root canal system and prevention of reinfection. However, due to the complex anatomy of the root canal system including lateral canals, accessory canals, and dentinal tubules—complete eradication of microorganisms remains challenging.[1,2]

Endodontic instruments, particularly K-files, come into direct contact with infected tissues during canal preparation and may act as potential carriers for microbial transmission if not adequately disinfected.[3] Endodontic-periodontal lesions frequently involve Porphyromonas gingivalis, a Gram-negative obligate anaerobe belonging to the red complex group. This pathogen is strongly associated with disease progression owing to its virulence factors such as fimbriae, lipopolysaccharides, and proteolytic enzymes, and has been detected in a substantial proportion of such lesions.[4,5]

NaOCl at 5.25% concentration has long been regarded as the gold standard disinfectant and irrigant in endodontics due to its broad-spectrum antimicrobial activity and tissue-dissolving properties. However, its use is limited by cytotoxicity to periapical tissues, potential for severe tissue irritation upon extrusion, unpleasant taste, and other adverse effects.[6] These drawbacks have prompted the exploration of biocompatible natural alternatives.

Garlic (Allium sativum) and its oil, rich in the bioactive compound allicin, have demonstrated antimicrobial, anti-inflammatory, and biocompatible properties against several oral pathogens. [7,8,11]

Although garlic oil has shown promising antimicrobial potential in dentistry, limited comparative data exist regarding its efficacy against Porphyromonas gingivalis on contaminated endodontic instruments. Therefore, the aim of this in vitro study was to evaluate and compare the antimicrobial efficacy of 5.25% sodium hypochlorite versus 100% garlic oil against Porphyromonas gingivalis on contaminated endodontic K-files.

The null hypothesis tested was that there is no statistically significant difference in the antimicrobial activity of 5.25% sodium hypochlorite and 100% garlic oil against Porphyromonas gingivalis under in vitro conditions. The alternative hypothesis was that there is a statistically significant difference between the two agents.

Materials and Methods

This in vitro experimental study was conducted in the "Department of Pediatric and Preventive Dentistry, in collaboration with "Maratha Mandal's Central Research Laboratory, Belgaum," after obtaining approval from the Institutional Ethics Committee (Ref. No.: 2025/PG/PEDO/1023).

Sample size and selection of materials: Sample size was calculated using two-means hypothesis testing (effect size = 0.89, α = 5%, power = 80%) based on previous similar studies. Although the minimum sample size required was 3 per subgroup, it was increased to 10 per subgroup to improve statistical power and reliability, resulting in a total sample of 40 files. Forty new stainless

©2026 IJDSIR, All Rights Reserved

steel #10 K-files (MANI Inc., Tochigi, Japan) were selected to ensure uniformity and eliminate pre-existing contamination.

Sterilization and contamination procedure: All files were placed in separate Eppendorf tubes and pre-sterilized using a standard autoclaving protocol at 121°C and 15 psi for 30 minutes. [8,9,10] Following sterilization, each file was aseptically transferred into a sterile Eppendorf microtube containing thioglycollate broth. A standardized suspension of Porphyromonas gingivalis (ATCC 33277) with a concentration of 10^5 CFU/mL was prepared, and 20 µL of the bacterial suspension was inoculated into each tube under aseptic conditions in a laminar airflow cabinet (Fig.1). The inoculated tubes were placed in an anaerobic jar system and incubated at 37°C for 8 days to allow adequate microbial colonization and biofilm formation on the file surfaces. Successful contamination was confirmed by visible turbidity in the broth.

Experimental groups and intervention: The contaminated files were randomly divided into four groups (n = 10 each) (Fig. 2A-D):

- Group IA: 5.25% NaOCl for 5 minutes
- Group IB: 5.25% NaOCl for 15 minutes
- Group IIA: 100% Garlic oil for 5 minutes
- Group IIB: 100% Garlic oil for 15 minutes

Each file was completely immersed in its respective test solution for the designated exposure time. "5.25% sodium hypochlorite (Prime Dental Products Pvt. Ltd., India) and 100% garlic oil (Salvia garlic oil – commercially available)" were used. After the exposure period, each file was aseptically removed, gently shaken to remove excess solution, and directly inoculated onto blood agar plates supplemented with hemin (5 mg/L) and vitamin K_1 (10 mg/L) using a standard streaking technique.

Microbiological assessment: The inoculated plates were incubated under anaerobic conditions at 37°C for 48 hours. Antimicrobial efficacy was evaluated by counting CFUs manually under a colony counter (Fig.3A -D). Plates showing no visible growth were recorded as zero CFUs.

Statistical analysis: Data were tabulated and analyzed using descriptive statistics (mean ± SD and median with interquartile range). Intergroup comparisons between 5.25% NaOCl and 100% garlic oil were performed using the Mann–Whitney U test. Intragroup comparisons at 5 min vs 15 min within each agent were performed using the Wilcoxon signed-rank test. A p-value < 0.05 was considered statistically significant. Analyses were carried out using SPSS software version 18.0 (IBM Corp., Armonk, NY, USA).

The study involved no human or animal participants; therefore, informed consent was not applicable. All laboratory procedures were performed under strict aseptic conditions following standard biosafety protocols

Results

Intergroup comparison revealed that NaOCl demonstrated significantly lower CFU counts than garlic oil at both 5-minute ($U = 3.500$, $Z = -3.523$, $p < 0.001$) and 15-minute intervals ($U = 0.000$, $Z = -3.781$, $p < 0.001$) (Tables 2 and 3). The mean and median CFU values were consistently lower in the NaOCl groups compared to the garlic oil groups at both time points (Table 1).

Intragroup analysis showed a statistically significant difference in CFU counts between 5- and 15-minute exposure periods for both NaOCl ($Z = -2.606$, $p = 0.009$) and garlic oil ($Z = -2.803$, $p = 0.005$) (Table 4).

CFU counts increased from 85.20 ± 20.917 to 138.90 ± 34.943 in the NaOCl groups and from 164.30 ± 46.562 to

©2026 IJDSIR, All Rights Reserved

278.10 ± 30.744 in the garlic oil groups between 5 and 15 minutes (Table 1).

No bacterial growth was observed in the negative control group, confirming the sterility of the experimental setup.

Discussion

The present in vitro study evaluated the antimicrobial efficacy of 5.25% sodium hypochlorite versus 100% garlic oil against Porphyromonas gingivalis on contaminated endodontic K-files, addressing the primary objective of direct comparison and the secondary objectives of assessing antimicrobial effectiveness under different exposure times".[1,2]

NaOCl demonstrated statistically significant antimicrobial efficacy compared with garlic oil at both 5-minute and 15-minute intervals.[1,2] This finding reinforces the established mechanism of NaOCl, which involves strong oxidizing capacity, chloramination reactions, and irreversible disruption of bacterial enzymes and cell wall integrity, thereby conferring broad-spectrum activity against anaerobic pathogens such as P. gingivalis.[3,14,15]

In contrast, 100% garlic oil exhibited moderate yet statistically significant antimicrobial activity.[1,2] The principal bioactive component, allicin, exerts its effect primarily through thiol-disulfide exchange reactions with sulfhydryl groups of essential bacterial enzymes, leading to metabolic inhibition and cell lysis.[4,16,17] This mechanism is supported by evidence that garlic derivatives inhibit key virulence factors of P. gingivalis, including gingipains.[5,6,18] Recent studies have further demonstrated garlic extract's inhibitory effects on periodontal pathogens, including reductions in protease activity and biofilm formation in planktonic and early biofilm models.[7,8,19]

The performance of NaOCl observed in this study is consistent with systematic reviews comparing herbal agents with NaOCl as endodontic disinfectants or irrigants, which generally conclude that natural alternatives exhibit lower antimicrobial potency against key endodontic-periodontal pathogens, although they may offer adjunctive value in biocompatibility-focused scenarios. [9,10,12,13,14,15]

While garlic oil showed lower efficacy compared to sodium hypochlorite, the observed antimicrobial activity supports its potential as a biocompatible adjunct, particularly in pediatric or regenerative contexts where cytotoxicity is a concern. Its additional anti-inflammatory and antioxidant properties may further contribute to a favourable safety profile.[12,19] However, the clinical relevance of these findings requires further investigation.

Limitations

The present study includes its strictly in vitro design using a single bacterial strain on K-files, which may not fully replicate the polymicrobial mature biofilm environment and complex anatomy of the root canal system. Variability in allicin concentration depending on preparation and storage and the characteristic odour of garlic oil may also influence clinical standardization and patient acceptability.

In summary, although 5.25% NaOCl demonstrated greater antimicrobial efficacy, 100% garlic oil exhibited measurable antimicrobial activity, indicating its potential as a natural adjunct in infection control strategies where biocompatibility is prioritized.

Conclusion

In conclusion, while 5.25% sodium hypochlorite remains the conventional disinfectant, 100% garlic oil demonstrated appreciable antimicrobial activity and may serve as a promising biocompatible natural adjunct for disinfecting endodontic instruments, particularly in pediatric patients where reduced cytotoxicity is desirable. Further studies using multi-species biofilm models and

©2026 IJDSIR, All Rights Reserved

ex vivo/in vivo designs are warranted to establish its clinical efficacy.

Clinical Significance

This study demonstrates that 100% garlic oil has significant antimicrobial activity against Porphyromonas gingivalis on contaminated endodontic K-files, although it is less effective than 5.25% NaOCl. In pediatric dentistry, garlic oil may offer a safer and more biocompatible alternative with reduced risk of tissue irritation and cytotoxicity. Its use could support effective infection control while enhancing patient safety during endodontic procedures.

Ethical approval: This study was approved by the Institutional Ethics Committee of the authors' institution (Ref. No. 2025/PG/PEDO/1023). Permission to conduct the microbiological procedures was obtained from the collaborating research laboratory.

Acknowledgement: The authors acknowledge the collaborating research laboratory for providing laboratory facilities and technical support for this study.

Abbreviations

Sodium hypochlorite (NaOCl)

Colony-forming units (CFUs)

References

1. Gomes BP, Jacinto RC, Pinheiro ET, Sousa EL, Zaia AA, Ferraz CC, et al. Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia and Prevotella nigrescens in endodontic lesions detected by culture and by PCR. Oral Microbiol Immunol. 2005;20(4):211-215.
2. Siqueira JF Jr, Rôças IN, Silva MG. Prevalence and clonal analysis of Porphyromonas gingivalis in primary endodontic infections. J Endod. 2008;34(11):1332-1336.
3. Yenni M, Bandi S, Avula SS, Margana PG, Kakarla P, Amrutavalli A. Comparative evaluation of four different sterilization methods on contaminated endodontic files. CHRISMED J Health Res. 2017;4(3):194-197.
4. Rafiei M, Kiani F, Sayehmiri K, Sayehmiri F, Tavirani MR, Dousti M, et al. Prevalence of anaerobic bacteria (Porphyromonas gingivalis) as major microbial agent in the incidence of periodontal diseases: a meta-analysis. J Dent (Shiraz). 2018;19(3):232-242.
5. Soman D, Nayak M, Radhakrishnan R, Kurup NB, CM M, Kurup N. Evaluation of the presence of Porphyromonas gingivalis and its correlation with signs and symptoms in endodontic-periodontal lesions: a cross-sectional study. Cureus. 2025;17(8).
6. Chaugule VB, Panse AM, Gawali PN. Adverse reaction of sodium hypochlorite during endodontic treatment of primary teeth. Int J Clin Pediatr Dent. 2015;8(2):153-156.
7. Shetty S, Thomas B, Shetty V, Bhandary R, Shetty RM. An in vitro evaluation of the efficacy of garlic extract as an antimicrobial agent on periodontal pathogens: a microbiological study. AYU. 2013;34(4):445-451.
8. Hugar S, Patel PM, Nagmoti J, Uppin C, Mistry L, Dhariwal N. An in vitro comparative evaluation of efficacy of disinfecting ability of garlic oil, neem oil, clove oil, and tulsi oil with autoclaving on endodontic K-files tested against Enterococcus faecalis. Int J Clin Pediatr Dent. 2017;10(3):283-288
9. Bakri IM, Douglas CWI. Inhibitory effect of garlic extract on oral bacteria. Arch Oral Biol. 2005;50(7):645-651
10. Widjaya AD, Amin MF, Aryadi, Roeslan BO. The effect of garlic extract (Allium sativum L.) (Amaryllidaceae) to eradicate the Porphyromonas

©2026 IJDSIR, All Rights Reserved

endodontalis biofilm: an in vitro research. Sci Dent J. 2021;5(3):138-143.

11. Isani A, Masih U, Joshi K. Ex vivo comparative evaluation of efficacy of disinfecting ability of garlic oil, neem oil, clove oil and tulsi oil with autoclaving on endodontic K-files tested against aerobic bacteria. Univ J Dent Sci. 2020;6(3):27-31.
12. Teja KV, Janani K, Srivastava KC, Shrivastava D, Jose J, Marya A, et al. Comparison of herbal agents with sodium hypochlorite as root canal irrigant: a systematic review of in vitro studies. Evid Based Complement Alternat Med. 2021;2021:8967219.
13. Susila AV, Sai S, Sharma N, Balasubramaniam A, Veronica AK, Nivedhitha S. Can natural irrigants replace sodium hypochlorite? A systematic review. Clin Oral Investig. 2023;27(5):1831-1849.
14. Cai C, Chen X, Li Y, Jiang Q. Advances in the role of sodium hypochlorite irrigant in chemical preparation of root canal treatment. Biomed Res Int. 2023;2023:8858283.
15. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes Infect. 1999;1(2):125-129.
16. Wallock-Richards D, Doherty CJ, Doherty L, Clarke DJ, Place M, Govan JRW, et al. Garlic revisited: antimicrobial activity of allicin-containing garlic extracts against Burkholderia cepacia complex. PLoS One. 2014;9(12).
17. Bhatwalkar SB, Mondal R, Krishna SBN, Adam JK, Govender P, Anupam R. Antibacterial properties of organosulfur compounds of garlic (Allium sativum). Front Microbiol. 2021;12:613077.
18. Bachrach G, Jamil A, Naor R, Tal G, Ludmer Z, Steinberg D. Garlic allicin as a potential agent for controlling oral pathogens. J Med Food. 2011;14(11):1338-1343.
19. Guan X, Zhou Y, Chen H, Wang C, Wang H. Antibacterial, anti-inflammatory, and anti-osteoclastogenesis roles of allicin in periodontitis. Int. J. Clin. Exp. Med. 2018 Jan 1;11(7):6721-6730.

Legend Figures and Tables

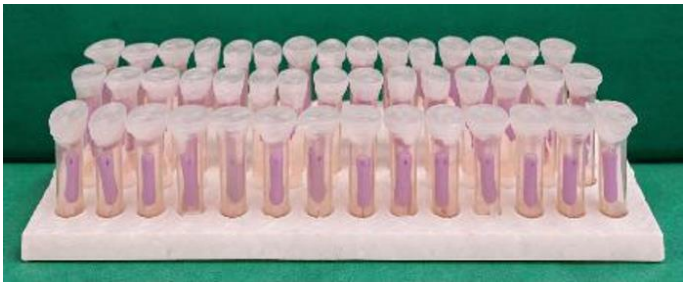


Figure 1: After sterilization each files were transferred to sterile Eppendorf tubes containing thioglycolate broth with Porphyromonas gingivalis suspension

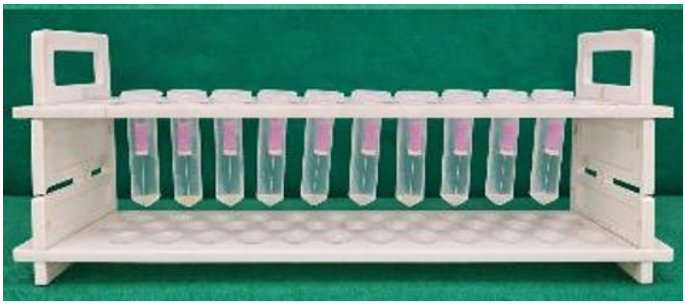


Figure 2A: Group IA: 5.25% NaOCl for 5 min



Figure 2B: Group IB: 5.25% NaOCl for 15 min

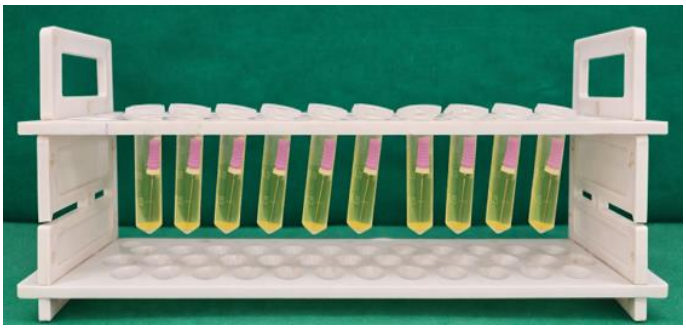


Figure 2C: Group II A: 100%Garlic oil 5 min

©2026 IJDSIR, All Rights Reserved

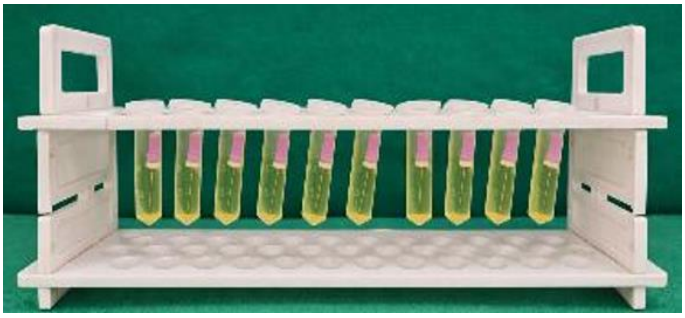


Figure 2D: Group II B: 100%Garlic oil 15 min

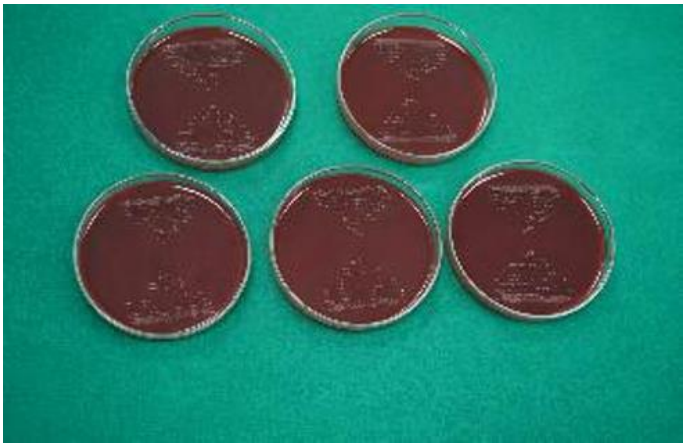


Figure 3A: Group I A CFU

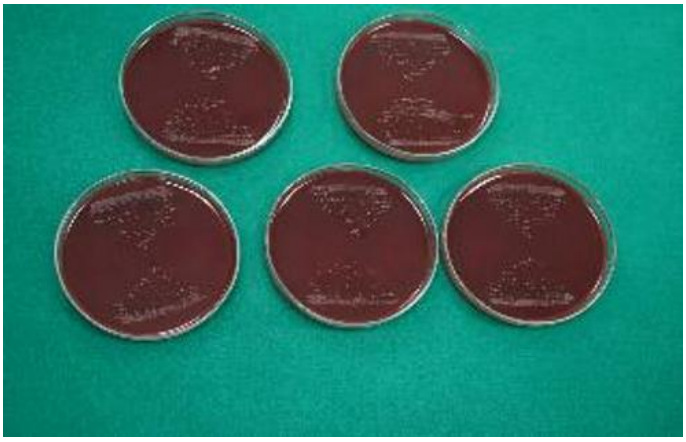


Figure 3B: Group I B CFU



Figure 3C: Group II A CFU



Figure 3D: Group II B CFU

Table 1: Mean ± SD and Median (Q1–Q3) of CFUs of Porphyromonas gingivalis in NaOCl and Garlic Oil Groups at Different Time Intervals

Sl. No.	Group	Mean ± SD	Median (Q1–Q3)
1	5.25% NaOCl – 5 min	85.20 ± 20.917	87.00 (66.50–106.50)
2	5.25% NaOCl – 15 min	138.90 ± 34.943	137.00 (108.75–166.00)
3	100% Garlic oil – 5 min	164.30 ± 46.562	159.00 (111.00–212.25)
4	100% Garlic oil – 15 min	278.10 ± 30.744	283.50 (258.25–298.50)

©2026 IJDSIR, All Rights Reserved

Table 2: Comparison of P. gingivalis count between 5.25% NaOCl and 100% garlic oil (5 minutes)

Sl No.	Groups Compared	U value	Z value	p-value
1	5.25% NaOCl vs 100% Garlic Oil	3.500	-3.523	< 0.001*

Table 3: Comparison of P. gingivalis count between 5.25% NaOCl and 100% garlic oil (15 minutes)

Sl No.	Groups Compared	U value	Z value	p-value
1	5.25% NaOCl vs 100% Garlic Oil	0.000	-3.781	< 0.001*

Table 4: Intragroup comparison of P. gingivalis count

Sl No.	Comparison	Z value	p-value
1	5.25% NaOCl (15 min vs 5 min)	-2.606	0.009*
2	100% Garlic Oil (15 min vs 5 min)	-2.803	0.005*

©2026 IJDSIR, All Rights Reserved