Antibacterial effectiveness of garlic (*Allium sativum Linn.*) extract nanoparticles against *E.faecalis*

Efektivitas antibakteri nanopartikel ekstrak bawang putih (*Allium sativum Linn.*) terhadap *E.faecalis*

Juni Jekti Nugroho, Tharisya Amiharna Kayla, Afniati Rachmuddin
Department of Conservative Dentistry, Faculty of Dentistry, Hasanuddin University, Indonesia
Corresponding author: **Juni Jekti Nugroho**, e-mail: **jektijuni@yahoo.co.id**

ABSTRACT
To determine the antibacterial effectiveness of 2.5% sodium hypochlorite (NaOCl) irrigation solution and garlic extract nanoparticles (*Allium sativum Linn.*) against *Enterococcus faecalis*. This type of research is an in vitro laboratory experiment with a post-test only with control group design using the dilution method. The research sample consisted of garlic nanoparticles, 2.5% NaOCl, and distilled water with each repetition carried out 3 times. The antibacterial ability is based on the inhibition zone formed around the stainless-steel cylinder on the MHA medium. Data processing and analysis techniques were carried out using the *Shapiro Wilk* test and one-way Anova. The average diameter of each inhibition zone for the antibacterial of garlic extract nanoparticles with concentrations of 20%, 30% and 40% was 30.69±1.03 mm, 34.73±1.09 mm and 39.66±2.14 mm, meanwhile 2.5% NaOCl showed an average inhibition zone diameter of 35.49±0.55 mm. It is concluded that garlic extract nanoparticles concentrations of 20%, 30% and 40% have a very strong antibacterial effect against *E.faecalis*.
Keywords: nanoparticles, garlic, antibacterial, *Enterococcus faecalis*

ABSTRAK
Untuk mengetahui efektivitas antibakteri larutan irigasi natrium hipoklorit (NaOCl) 2,5% dan nanopartikel ekstrak bawang putih (*Allium sativum Linn*.) terhadap *Enterococcus faecalis*. Penelitian eksperimen laboratorik in vitro dengan *post-test only with control group design*, dengan metode dilusi. Sampel terdiri dari nanopartikel bawang putih, NaOCl 2,5%, dan akuades dengan masing-masing pengulangan sebanyak 3 kali. Kemampuan antibakteri didasarkan pada zona hambat yang terbentuk di sekitar silinder *stainless steel* pada media MHA. Teknik pengolahan dan analisis data dilakukan dengan menggunakan uji *Shapiro Wilk* dan Anova satu arah. Rerata diameter zona hambat antibakteri nanopartikel ekstrak bawang putih 20%, 30%, dan 40% adalah 30,69±1,03 mm, 34,73±1,09 mm, dan 39,66±2,14 mm. Sedangkan NaOCl 2,5% menunjukkan rerata diameter zona hambat sebesar 35,49±0,55 mm. Disimpulkan bahwa nanopartikel ekstrak bawang putih konsentrasi 20%, 30% dan 40% memiliki efek antibakteri yang sangat kuat terhadap *E.faecalis*.
Kata kunci: nanopartikel, bawang putih, antibakteri, *Enterococcus faecalis*
Received: 10 January 2024 Accepted: 1 April 2024 Published: 1 August 2024

INTRODUCTION

Bacteria are the main etiology of endodontic infections. After the pulp is exposed due to caries, bacteria occupying the pulp space and root canal lumen will invade the entire root canal system, causing intra-radicular infection.[1,2] According to Alghamdi and Shakir,[3] cases with pain and infection following endodontic treatment are predominantly associated with the presence of *Enterococcus faecalis*, with a high prevalence rate reaching 90% in all cases. The *E.faecalis* is a Gram-positive facultative anaerobic bacterium that causes opportunistic infections. This bacterium possesses numerous virulence factors that enable it to survive in unfavorable conditions, such as low oxygen environments, temperatures between 10-60 °C, high salinity, high pH, or nutrient-deprived environments. Based on these virulence factors, *E.faecalis* can contribute to failures in endodontic treatment.[4]

Endodontic treatment aims to eliminate bacteria from the root canal system and prevent secondary infections. The debridement phase becomes challenging due to the complexity of root canal anatomy and the presence of persistent bacteria. Therefore, irrigation solutions are needed to complement the mechanical action of debridement, ensuring the maximal elimination of bacteria.[5] Sodium hypochlorite (NaOCl) is the most widely used irrigation solution. This solution produces acid and hypochlorite ions that act as antibacterial agents. Cytotoxicity and caustic effects of NaOCl on healthy peri-radicular tissues during unintentional extrusion in irrigation procedures are reasons for using lower concentrations, typically between 0.5-1% or 2.5%. However, at these concentrations, NaOCl is not capable of removing the inorganic components of the smear layer.[6,7]

The limitations of NaOCl irrigation solution and to mitigate its side effects, therefore this research approach focuses on the use of natural substances. One natural substance with antibacterial properties is garlic (*Allium sativum Linn.*) because it contains *alliin* (*S-allyl cysteine sulfoxide*), which is converted into *allicin* and several organosulfur components by the enzyme *alliinase* when garlic is crushed. Dried garlic powder contains approximately 1% *alliin*. The extract content demonstrates a broad-spectrum inhibitory effect on the growth of various Gram-positive and Gram-negative bacteria.[8,9]

The study by Birring *et al,*[10] on the effectiveness of 70% concentration garlic extract demonstrated the most effective results in preventing biofilm formation by *E.faecalis*. Another study by Octavia *et al*[11], proved that garlic extract concentrations of 25%, 50% and 100% could reduce the viability of *E.faecalis*. However, the concentrations of garlic extract used in these studies were still very high, potentially leaving sediment in the root canal.

Nanoparticle technology is widely used in biomedicine, particularly in addressing the challenges of poorly soluble active substances and low bioavailability.[12] The formulation of garlic extract nanoparticles aims to reduce the normal dose of garlic extract. Additionally, garlic has a strong odor, lipophilic properties, high volatility, and low physicochemical stability, limiting its extensive use. This technology is an alternative expected to enhance func-

DOI 10.35856/mdj.v13i2.921

tional properties and be more advantageous.[13] Gabriel *et al*[14] demonstrated formation of inhibition zones against the growth of *S.aureus*, *E.coli*, *S.mutans*, and *P.gingivalis* by 40% and 60% garlic extract nanoparticles, indicating a strong bacterial growth inhibitory effect. Based on the background, the author aims to evaluate the antibacterial effectiveness of garlic nanoparticle extract formulation against *E.faecalis*.

METHOD

The research was validated by Ethics and Advocacy Unit of Hasanuddin University (No.0142/PL.09/KEPK FKG-RSGM UNHAS/2023). This study utilizes garlic processed into an extract through mechanical method using a blender with aquadest as the solvent. The garlic extract is reduced to the nanometer scale and precipitated to obtain a mass in the form of garlic extract nanoparticles.

The samples used in this study are *E.faecalis* bacteria. The determination of the minimum inhibitory concentration (MIC) is conducted by observing the colony count on petri dishes. Subsequently, the treatment groups are divided into 5 categories, which are aquadest as the negative control, 2.5% NaOCl solution as the positive control, and test groups using garlic extract nanoparticles at concentrations of 20%, 30% and 40%. The materials used in this study include garlic, 2.5% NaOCl, *E.faecalis* ATCC 29212, Mueller Hinton Agar (MHA), blood agar (BA), brain heart infusion broth (BHIB), and aquadest solution.

The petri dishes are divided into 5 gradients. Following the principles of the dilution method, each gradient will contain a sterile stainless-steel cylinder, each pretreated with aquadest as the negative control, a 2.5% NaOCl solution as the positive control, and test groups using garlic extract nanoparticles at concentrations of 20%, 30% and 40%. Subsequently, *E.faecalis* bacteria are added to each petri dish, poured along with MHA media between the stainless-steel cylinders in each petri dish. Three petri dishes containing samples are then incubated for 24 hours at a temperature of 37°C. After the incubation is complete, the inhibition zones formed around the stainless-steel cylinders are observed, and their diameters are measured in millimeters using a digital caliper.

RESULT

The MIC test in this study is conducted by observing the colony count on petri dishes. The concentrations of garlic extract nanoparticles used are 10%, 20% 30% and 40%, as indicated in Fig.1. After incubating for 24 hours, the obtained results indicate that the MIC capable of inhibiting the growth of *E.faecalis* is 30% concentration of garlic extract nanoparticles.

After obtaining the MIC values, the inhibitory power test was continued using the dilution method with stainless steel cylinders in five treatment groups, namely positive control (2.5% NaOCl), aquadest as the negative control, garlic extract nanoparticles at 20%, 30%, and 40% concentrations, with the results as stated clearly in Table 1.

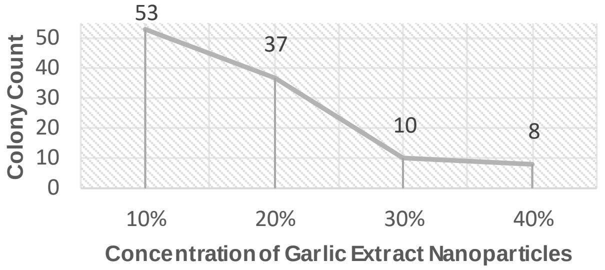

Figure 1 Graph of *E.faecalis* quantity (x 10^8 CFU/mL) at MIC

Table 1 Mean values and standard deviations of the diameter of inhibition zones for *E.faecalis* bacteria after 24 hours.

Concentration	N	Inhibition Zone		
		Mean ± SD	Min	Max
Aquades	3	0,00	0.00	0.00
Garlic extract 20%	3	30.69±1.03	29.20	32.70
Garlic extract 30%	3	34.73±1.09	33.10	36.80
Garlic extract 40%	3	39.66±2.14	36.40	42.00
NaOCl 2.5%	3	35.49±0.55	34.60	36.40

N=sample size *Min*=smallest diameter of the inhibition zone
SD=standard deviation *Max*=largest diameter of the inhibition zone
Mean=average diameter of the inhibition zone

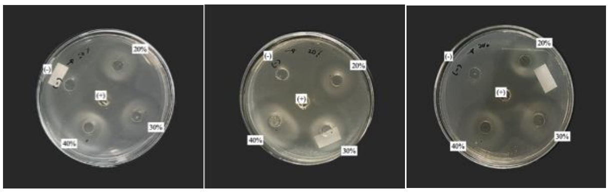

Figure 2 Inhibition zones of *E.faecalis* bacteria

Table 2 Results of the *Shapiro-Wilk* and *one-way* Anova statistical tests for the inhibition zones of *E.faecalis* bacteria.

Concentration	N	Inhibition Zone	
		Normality test*	***Comparison test*****
Aquades	3	-	
Garlic extract 20%	3	0.107*	
Garlic extract 30%	3	0.698*	0.344**
Garlic extract 40%	3	0.286*	
NaOCl 2.5%	3	0.956*	

Based on Table 1, it is observed that all test groups have values for the inhibition zone, except for the aquadest test group. According to Buldani *et al*[23], the antibacterial strength of garlic extract nanoparticles at concentrations of 20%, 30% and 40% is considered very strong (>20 mm) with average diameter values of the inhibition zone being 30.69±1.03 mm, 34.73±1.09 mm, and 39.66±2.14 mm, respectively. Additionally, the antibacterial strength of garlic extract nanoparticles at a concentration of 40% shows a greater value compared to 2.5% NaOCl, with an average diameter of the inhibition zone being 35.49±0.55 mm. The inhibition zone calculations were conducted on petri dishes as indicated in Fig.2.

Next, the *Shapiro-Wilk* test was conducted to assess the normality of the data, followed by the one-way Anova statistical test to compare the data sets (Table 2). Based on the Shapiro-Wilk statistical test, a p-value of (p>0.05) was obtained, indicating that the data is normally distributed. Additionally, based on the one-way Anova statistical test, a value of 0.344 (p>0.05) was obtained, indicating no significant difference between the data sets of the treatment groups. The test results suggest that there is no need to conduct the LSD statistical test because the Anova results indicate nonsignificant findings.

DISCUSSION

The *E.faecalis* is a bacterium commonly found in the root canal and can survive in a pH range of 4-11, as well as temperatures between 10-45°C. Prolonged root canal infections allow *E.faecalis* to invade the entire root canal system, including branches and dentin tubules.[15,16] Additionally, this species has the ability to persist in the root canal even under extreme conditions.[17]

Endodontic irrigation plays a crucial role in root canal debridement and is considered the only way to clean limited areas that cannot be reached by mechanical means. The extensive wall area and anatomical complexity of the root canal result in the walls remaining untouched, regardless of the instrumentation technique used. Due to its antibacterial properties and its ability to dissolve organic materials, NaOCl is the most popular irrigating solution used in dentistry.[18] Ideally, an irrigating material should be biocompatible with tissues, but NaOCl can alter the elastic modulus, tensile and flexural strength, as well as the microhardness of dentin. Additionally, it can be caustic if accidentally extruded apically.[19]

In this study, the garlic extract nanoparticles were obtained through an extraction process using aquades as the solvent.[20] The primary advantage of extraction with aquades is the potential for consumption or direct administration to animals or in vitro cells without additional processing. Bajac *et al,*[21] evaluated the bioactive content of garlic extract using aquades and ethanol extraction methods, and it was found that aquades extraction resulted in the highest content of *allicin*, *allyl sulfide*, and *methanethiosulfonic acid S-methyl ester*. Phytochemical analysis of aquades extraction revealed the presence of bioactive substances such as tannins, terpenoids, and saponins in moderate quantities, as well as low quantities of flavonoids and alkaloids. These components are responsible for the antibacterial properties of garlic.[22]

This study is a laboratory experimental research aimed to determine the antibacterial effects of garlic extract nanoparticles at concentrations of 20%, 30%, and 40% against *E.faecalis*. The three concentrations were chosen based on the results of the MIC test and the effective concentration was found to be 30%. In the inhibitory power test conducted at concentrations of 30% and 40%, the inhibition zones formed were 34.73 ± 1.09 mm and 39.66 ± 2.14 mm, respectively. According to Buldani *et al,*[23] inhibition zones exceeding 20 mm are considered very strong. This indicates that the used concentrations are capable of inhibiting *E.faecalis*, and as the concentration of garlic. increases, the inhibition zone for *E.faecalis* also increases. The bioactive compound (*allicin*) obtained when garlic is crushed inhibits the proliferation and induces the death of *E.faecalis* bacteria.[24,25] Furthermore, the lipophilic nature of allicin affects the structure and integrity of the phospholipid membrane of *E.faecacalis* bacteria, causing cellular content leakage.[26] These results align with the study by Octavia *et al*, which examined the viability of *E.faecalis* against garlic extract at concentrations of 25%, 50% and 100%, finding viabilities of 5.17%, 4.71% and 4.62%, respectively.[11] Another study by Soraya *et al*[27] also showed an increase in the effectiveness of garlic extract with increasing concentration.

Birring *et al*[10] investigated garlic extract concentrations of 10%, 40% and 70% using the diffusion method and found that the most effective concentration in inhibiting *E.faecalis*. Soraya *et al*[27] also examined garlic extract concentrations of 12.5%, 25%, 50%, 75% and 100%, and they found that a concentration of 75% inhibited *E.faecalis* more effectively compared to concentrations of 12.5%, 25% and 50%. In contrast, in the MIC test of this study, the MIC of garlic extract nanoparticles was found to be at a concentration of 30%. These results indicate that the use of garlic extract in nanoparticle form has antibacterial effects at lower concentrations compared to previous studies.

The calculation of the average inhibition zones indicates a comparison between treatment groups, showing that 2.5% NaOCl is more effective compared to 30% garlic extract nanoparticles. However, at a concentration of 40%, garlic extract nanoparticles demonstrate better effectiveness compared to 2.5% NaOCl in inhibiting the growth of *E.faecalis*. In the study by Ghoddusi *et al,*[28] the results showed that garlic extract was equally effective as 2.5% NaOCl against *E.faecalis* at a concentration of 70%. The findings of this study suggest that garlic extract nanoparticles are statistically more effective than 2.5% NaOCl in inhibiting the growth of *E.faecalis* at much lower concentrations compared to previous research. This could be attributed to the homogenous distribution of nanoparticle formulations and their good solubility and penetration capabilities, allowing them to penetrate bacterial cells faster and kill more bacteria. This aligns with the characteristics of nanoparticles, which exhibit high efficiency even at low concentrations.[29]

Based on the results of this study, it can be seen that the utilization of nanoparticle technology can be a solution to enhance the antibacterial effects of natural substances. However, it's important to note that higher nanoparticle sizes and concentrations can lead to increased precipitation and agglomeration of the material.[30]

It is concluded that garlic extract nanoparticles at concentrations of 20%, 30% and 40% exhibit antibacterial effects against *E.faecalis*. The effectiveness of garlic extract nanoparticles at concentrations of 40% only is higher than NaOCl 2.5%. However, there is no significant difference in inhibiting the growth of *E.faecalis*.

REFERENCES

1. Mintrakul K, Vongsawan K, Watcharakirin W, Khererat P. Quantitative analysis of *Lactobacillus* and *Enterococcus faecalis* between irreversible pulpitis and pulp necrosis in primary teeth. Dent Res Oral Health. 2019;2(2):17.

DOI 10.35856/mdj.v13i2.921

2. Zargar N, Marashi MA, Ashraf H, Hakopian R, Beigi P. Identification of microorganisms in persistent/secondary endodontic infections with respect to clinical and radiographic findings: Bacterial culture and molecular detection. Iranian J Microbiology. 2019;11(2): 121.
3. Alghamdi F, Shakir M. The influence of *Enterococcus faecalis* as a dental root canal pathogen on endodontic treatment: A systematic review. Cureus. 2020;12(3): e7257. doi:10.7759/cureus.7257.
4. Bajoria AA, Alfawzan AA, Ramaiah VV, Habibullah MA, Tareen SU, Babaji P. Evaluation of pathological microbes in root canals with pulp necrosis. World J Dent 2020;11(3): 211.
5. Siddique R, Anjaneyulu K, Muralidharan NP. Antimicrobial efficacy of garlic-lemon in comparison with sodium hypochlorite against *E.faecalis*. J Clin Diagn Res. 2020;13: 57.
6. Gopikrishna V. Grossman's endodontic practice. 14th ed. New Delhi: Wolters Kluwer Health; 2021. p.298-301.
7. Hajihassani N, Alavi O, Karamshahi M, Marashi SM, Khademi A, Mohammadi N. Antibacterial effect of nano-chlorhexidine on *Enterococcus faecalis* biofilm in root canal system: An in vitro study. J Dent Res. 2022;19: 80.
8. Shooriabi M. Effects of *Allium sativum* (garlic) and its derivatives on oral diseases: A narrative review. J Res Dent Maxillofac Sci 2021;6(1): 37.
9. Prabhakaran P, Mariswamy AB. A scanning electron microscope evaluation of efficacy of sodium hypochlorite and *Allium sativum* in smear layer removal in root canals with the use of modified evacuation system: An ex vivo study. J Conserv Dent 2018;21: 401-7.
10. Birring OJ, Viloria IL, Nunez P. Anti-microbial efficacy of *Allium sativum* extract against *Enterococcus faecalis* biofilm and its penetration into the root dentin: An in vitro study. Indian J Dent Res. 2015;26: 477.
11. Octavia A, Budiardjo SB, Indiarti IS, Fauziah E, Suharsini M, Sutadi H, *et al*. Garlic extract efficacy against the viability of *Enterococcus faecalis* (In vitro). Int J Appl Pharmaceut 2019;11:194.
12. Kumar M, Curtis A, Hoskins C. Application of nanoparticle technologies in the combat against anti-microbial resistance. Pharmaceut 2018;10(1):11.
13. Khrisna SBN. Emergent roles of garlic-based nanoparticles for bio-medical applications - A review. Current Trends in Biotechnol Pharm 2021;15(3): 350.
14. Gabriel T, Vestine A, Kim KD, Kwon SJ, Sivanesan I, Chun SC. Antibacterial activity of nanoparticles of garlic (*Allium sativum*) extract against different bacteria such as *Streptococcus mutans* and *Phorpyromonas gingivalis*. Appl Sci 2022;12: 3491. doi:10.3390/app12073491.
15. Mahendra I, Wardani I, Rochyani L. Daya antibakteri ikan teri jengki (*Stolephorus insularis*) terhadap *Enterococcus faecalis*. Jurnal Kedokteran Gigi 2018; 12(2): 106-16.
16. Asmah N. Pathogenicity biofilm formation of *Enterococcus faecalis*. Journal of Syiah Kuala Dentistry Society 2020;5(1): 478.
17. Anisa PDN, Prisinda D. Perawatan saluran akar ulang non-bedah gigi insisivus lateral kanan rahang atas pada pasien geriatri. Jurnal Kedokteran Gigi 2020; 32(3): 232-43.
18. Cîmpean SI, Pop-Ciutrila IS, Matei SR, Colosi IA, Costache C, Nicula GZ, *et al*. Effectiveness of different final irrigation procedures on *Enterococcus faecalis* infected root canals: An in vitro evaluation. Materials 2022; 15: 6688. doi: 10.3390/ma 15196688.
19. Boutsioukis C, Arias-Moliz MT. Present status and future directions-irrigants and irrigation methods. Int Endod J. 2022;55: 588-612. doi: 10.1111/iej.13739.
20. Bar M, Binduga UE, Szychowski KA. Methods of isolation of active substances from garlic (*Allium sativum L*.) and its impact on the composition and biological properties of garlic extracts. Antioxidants 2022;11:1345. doi: https://doi.org/10.3390/antiox11071345.
21. Bajac J, Nikolovski B, Kocic-Tanackov S, Tomšik A, Mandic A, Gvozdanovic-Varga J, *et al*. Extraction of different garlic varieties (*A. sativum L*.) determination of organosulfur compounds and microbiological activity. In Proceedings of the IV International Congress Food Technology, Quality and Safety, Novi Sad, Serbia, 23-25 October 2018; p.82.
22. Johnson M, Olaleye O, Kolawole O. Antimicrobial and antioxidant properties of aqueous garlic (*Allium sativum*) extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Br Microbiol Res J 2016; 14: 1-11.
23. Buldani A, Yulianti R, Soedomo P. Uji efektivitas ekstrak rimpang bangle (*Zingiber cassumnar Roxb*.) sebagai antibakteri terhadap *Vibrio cholerae* dan *Staphylococcus aureus* secara *in vitro* dengan metode difusi cakram. Senit 2017;2: 230-1
24. Sarfraz M, Nasim MJ, Jacob C, Gruhlke MCH. Efficacy of *allicin* against plant pathogenic fungi and unveiling the underlying mode of action employing yeast based chemogenetic profiling approach. Appl Sci 2020;10:2563. doi:10.3390/app10072563.
25. Kristiananda D, Allo JL, Widyarahma VA, Lusiana, Noverita JM, Riswanto FDO, *et al*. Aktivitas bawang putih (*Allium sativum L*.) sebagai agen antibakteri. Jurnal Ilmu Farmasi dan Farmasi Klinik. 2022;19(1): 46-9.
26. Magryś A, Olender A, Tchórzewska D. Antibacterial properties of *Allium sativum L*. against the most emerging multidrug-resistant bacteria and its synergy with antibiotics. Arch Microbiol 2021; 203: 2257-68. doi: https://doi.org/10.1007/s00203-021-02248-z.
27. Soraya C, Chismirina S, Novita R. Influence of garlic juice (*Allium sativum L*.) as root canal irrigation material to inhibit the growth of *Enterococcus faecalis* in vitro. Cakradonya Dent J. 2018;10(1): 1-9.
28. Ghoddusi J, Forghani M, Bagheri H, Aryan E, Koohi Noghondar M, Hajizadeh S. Antibacterial efficacy of *Allium sativum L*. extract as a root canal irrigant in pulpless teeth with infected root canal systems. Int J Res Report Dent 2021; 4(4):147-55.
29. Syahrial S, Rimbawan R, Damayanti E, Astuti DP, Suptijah P. Pengaruh pemberian nano daun kelor (*Moringa oleifera*) terhadap kadar mineral serum dan tulang pada tikus *sprague dawley* jantan tumbuh. Indonesian J Nutrition 2019;7(2):114-20. doi: 10.14710/jgi.7.2.114-120.
30. Algharib SA, Dawood A, Zhou K, Chen D, Li C, Meng K, *et al*. Preparation of chitosan nanoparticles by ionotropic gelation technique: Effects of formulation parameters and in vitro characterization. J Molec Struct 2022; 1252:132129. doi: https://doi.org/10.1016/j.molstruc.2021.132129.