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Antibacteria Susceptibility Test of Garlic (Allium sativum L.) Against Enterococcus faecalis As Root Canal Medicament

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KEYWORDS

Garlic, *Enterococcus faecalis*, antibacterial, well diffusion, endodontic

ABSTRACT

This study aims to investigate the effect of garlic extract (GE) on the inhibition of *Enterococcus faecalis* (EF) bacterial growth. This study is a laboratory experimental research with a true experimental post-test only control group design, conducted in vitro using the well diffusion method. The experimental group consists of EF bacteria treated with different concentrations of GE, while the control group consists of EF bacteria treated with 0.2% chlorhexidine (CHX) as the positive control (PC) and 10% dimethyl sulfoxide (DMSO) as the negative control (NC). This study aims to observe the inhibition zone diameter (IZD) of bacterial growth after treatment with different concentrations of GE or control agents, measured in millimeters using a caliper. Phytochemical test revealed that the GE used in this study contains secondary metabolite compounds such as phenolics, flavonoids, saponins, and steroids, resulting in an IZD of 5.85 ± 0.26 at 100% GE and 3.25 ± 0.27 at 75% GE. The IZD formed on PC was 17.34 ± 0.33, and no IZD was formed on 50% GE or NC. The effect of GE in inhibiting the growth of EF was observed by the presence of inhibition zones at 100% and 75% concentrations, although it was not yet able to match the effectiveness of 0.2% CHX.

INTRODUCTION

Root canal treatment is an endodontic procedure that aims to clean out the pulp tissue or microorganisms present within the root canal system, allowing for proper root canal filling and periapical tissue repair (Gomes et al., 2023). Root canal treatment can also fail, and the causes of root canal treatment failure include incomplete obturation, overfilling, periapical cysts, fractured instruments left in the root canal, and microorganisms (Gulabivala & Ng, 2023). EF is a facultative anaerobic Gram-positive bacteria that can form biofilms that allow bacteria to resist phagocytosis, antibodies, and antimicrobials (Oli et al., 2022). EF has the potential to be a virulence factor that can survive in extreme environmental conditions and is also resistant to some antimicrobials used in root canal treatment (Elashiry et al., 2023).

Managing post-root canal treatment failure caused by EF requires microbial control by inhibiting these bacteria with various antibacterial drugs (Alghamdi & Shakir, 2020). Root canal sterilization is the process of eliminating pathogenic microorganisms using root canal medicaments (Zou et al., 2024). Before sterilization, biomechanical preparation is performed as an effective procedure for removing microorganisms (Lakshmanan & Jeevanandan, 2022).

However, this procedure cannot completely eliminate bacteria, necessitating intracanal medicaments to further reduce bacteria within the root canal system (Ratih et al., 2022).

Root canal medicaments commonly include phenol derivatives such as formocresol, camphorated parachlorophenol, eugenol, metacresylacetate, and halides (iodine-potassium iodide) (Wozniak et al., 2015). These medications exhibit antigenic and cytotoxic properties but have limited short-term efficacy (Bhandi et al., 2022). CHX, for instance, can stain teeth and be toxic with regular use (Brookes et al., 2020). Natural materials like garlic are currently being explored as root canal medicaments. Studies have demonstrated garlic's ability to inhibit bacterial growth, including EF (Soraya et al., 2018).

Garlic (*Allium sativum* L.) is a natural plant with a long history of culinary and medicinal use (Tesfaye, 2021). Garlic (*Allium sativum* L.) has been reported to possess various biological properties, including antimicrobial, anticancer, antioxidant, immunomodulatory, anti-inflammatory, and hypoglycemic effects (Beshbishy et al., 2020). The antimicrobial activity of garlic can be attributed to its active compounds (Gabriel et al., 2022). Previous studies investigating the effect of GE on *Streptococcus mutans* growth demonstrated its ability to inhibit bacterial growth . Another study examining the effect of GE on EF growth, using CHX as a PC, showed effective inhibition at a concentration of 75% with IZD of 17 mm, while at 25%, the IZD was 11 mm, representing the smallest diameter.

Based on the foregoing discussion, this study aims to investigate the effect of GE on the inhibition of EF bacterial growth.

Several previous studies have explored the effects of garlic extract (Allium sativum) on bacterial growth, including Enterococcus faecalis (EF). Soraya et al. (2018) reported that garlic juice is effective in inhibiting the growth of EF in vitro, demonstrating significant zones of inhibition at certain concentrations. This study emphasizes the potential of garlic as an irrigating agent in root canal treatment.

Furthermore, research by Bhatwalkar et al. (2021) showed that organosulfur compounds from garlic have strong antibacterial activity against various bacterial strains, including oral pathogenic bacteria. The findings of this research provide evidence that garlic not only has culinary properties but also medicinal benefits, making it an attractive candidate as an antibacterial agent in dental practice.

Gabriel et al. (2022) in their study on nanoparticles of garlic extract found that these compounds showed better antibacterial activity against Streptococcus mutans and Porphyromonas gingivalis compared to positive controls. This research indicates that the combination of garlic extract with nanoparticle technology can enhance antibacterial effectiveness, opening opportunities for broader clinical applications.

On the other hand, research by Aftab et al. (2022) focused on the use of other antibacterial agents in managing bacterial infections. Although this study provides new insights into the use of various antibacterial drugs, there is still a need to explore natural materials like garlic as safer and more effective alternatives.

Despite the various studies on the antibacterial activity of garlic, there is still a lack of understanding regarding the specific mechanisms of the active compounds contained in garlic extract and the optimal concentrations needed to effectively inhibit the growth of EF. Research that focuses specifically on the efficacy of different concentrations of garlic extract and its comparison with conventional antibacterial agents like chlorhexidine is still rare. This presents an opportunity to address the question of how far garlic can function as an effective alternative in endodontic treatment.

The urgency of this research lies in the increasing resistance of bacteria to conventional antibiotics used in dental health care. Several microorganisms, including Enterococcus

faecalis, have shown the ability to withstand various commonly used antibacterial agents. In this context, the exploration of natural materials such as garlic as therapeutic alternatives becomes very important. Considering the widely recognized antiseptic properties of garlic, this research is expected to offer a safer and more effective solution in managing bacterial infections in the root canal.

The novelty of this study is the systematic approach to exploring the effects of garlic extract on Enterococcus faecalis growth by analyzing its effectiveness at various concentrations. This study also incorporates phytochemical analysis to identify the active compounds contributing to antibacterial activity, which has not been widely done in previous studies. Thus, this research not only provides evidence that garlic can function as an antibacterial agent but also elucidates the potential mechanisms behind its effectiveness.

The objective of this study is to evaluate the effectiveness of garlic extract as an antibacterial agent against the growth of Enterococcus faecalis using the well diffusion test method. Additionally, this research aims to determine the optimal concentration of garlic extract that provides the most significant inhibitory effect against bacterial growth.

This study is expected to provide useful information for dental practitioners in selecting safer and more effective root canal irrigating agents. With solid scientific evidence regarding the effectiveness of garlic extract in inhibiting bacterial growth, it is anticipated to reduce the use of synthetic antibiotics that may contribute to resistance and side effects. Furthermore, the results of this research can serve as a foundation for further studies focused on utilizing natural materials in the management of bacterial infections in the medical context.

RESEARCH METHOD

Study Design

This study is a laboratory experimental research with a true experimental post-test only control group design, conducted in vitro using the well diffusion method. The experimental group consists of EF treated with different concentrations of GE, while the control group consists of EF treated with 0.2% CHX as the PC and 10% DMSO as the negative NC. This study aims to observe the IZD of bacterial growth after treatment with different concentrations of GE or control agents, measured in millimeters using a caliper.

Sample Size Calculation

The sample size for this study was calculated using Federer's formula (Soesanto et al., 2023). Based on the Federer's formula calculation, the minimum number of replications for each treatment is five.

Materials Collection and Preparation

This research utilizes garlic (*Allium sativum* L.) obtained from the Balai Pengujian Standar Instrumen Tanaman Sayuran, West Bandung Regency. The acquired garlic is cleaned with water, thinly sliced, and dried using wind without exposure to sunlight. The dried garlic is then blended until fine, weighed to 250g, and extracted using the maceration method with 96% ethanol solvent for 500ml over 24 hours, repeated three times. The macerate is filtered using filter paper and evaporated using a rotary evaporator at 50rpm and 40°C to obtain concentrated GE. The concentrated GE is then diluted with 10% DMSO at concentrations of 50%, 75%, and 100%. The extracted garlic is then subjected to qualitative phytochemical tests.

Sample Preparation

This study employs EF ATCC 29212 as the bacterial sample. Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB) are heated to boiling in a microwave until homogeneous and then sterilized in an autoclave at 121°C for 20 minutes. MHA is poured into petri dishes to form agar plates, and EF colonies cultured in MHA are inoculated into MHB.

The suspension is homogenized using a vortex mixer until it reaches a turbidity equivalent to the McFarland standard 0.5 or $1.5x10^8$CFU/ml.

Antimicrobial Susceptibility Test

A sterile cotton swab is dipped into the MHB containing the EF suspension and evenly spread over the surface of the MHA agar plate. The plate is allowed to stand at room temperature for 3-5 minutes until the suspension is absorbed into the agar. Five 6mm wells are made in the MHA using a sterile tip and filled with 50µl of GE 100%, GE 75%, GE 50%,PC, and NC. The agar plates are incubated at 37°C for 24 hours, allowing clear inhibition zones to develop. These zones are measured using a caliper in millimeters. The IZD is calculated by subtracting the diameter of the well from the diameter of the clear zone. This procedure is repeated five times for each treatment.

RESULT AND DISCUSSION

Based on the phytochemical tests, phenolic compounds, flavonoids, saponins, and steroids were identified in the GE used in this study. The qualitative phytochemical test results are summarized in **Table 1**.

Table 1. Results of phytochemical tests of GE

No	Secondary Metabolite Compound	Test Method	Result
1	Phenolic	5% $FeCl_3$	+
2	Tannin	1% $FeCl_3$	-
3	Flavonoid	Concentrated HCl + Mg	-
		2N H_2SO_4	-
		10% NaOH	+
4	Saponin	Boiled	+
5	Triterpenoid	Concentrated H_2SO_4 + CH_3COOH anhydrous	-
6	Steroid	Concentrated H_2SO_4 + CH_3COOH anhydrous	+
7	Alkaloid	Dragendorff	-

In this study, antibacterial activity was evaluated by measuring the inhibition zone diameters (IZD) of bacterial growth following treatment with five different agents. The IZD was determined using a caliper and measured in millimeters. The area measured was the clear zone (inhibition zone) where EF were absent. The results revealed that PC treatment produced an average IZD of 17.34mm (strong), GE 100% treatment resulted in an average IZD of 5.85mm (moderate), and GE 75% treatment yielded an average IZD of 3.25mm (weak). No IZD was observed with NC and GE 50% treatments. The antibacterial activity results are presented in **Figure 1** and **Figure 2**.

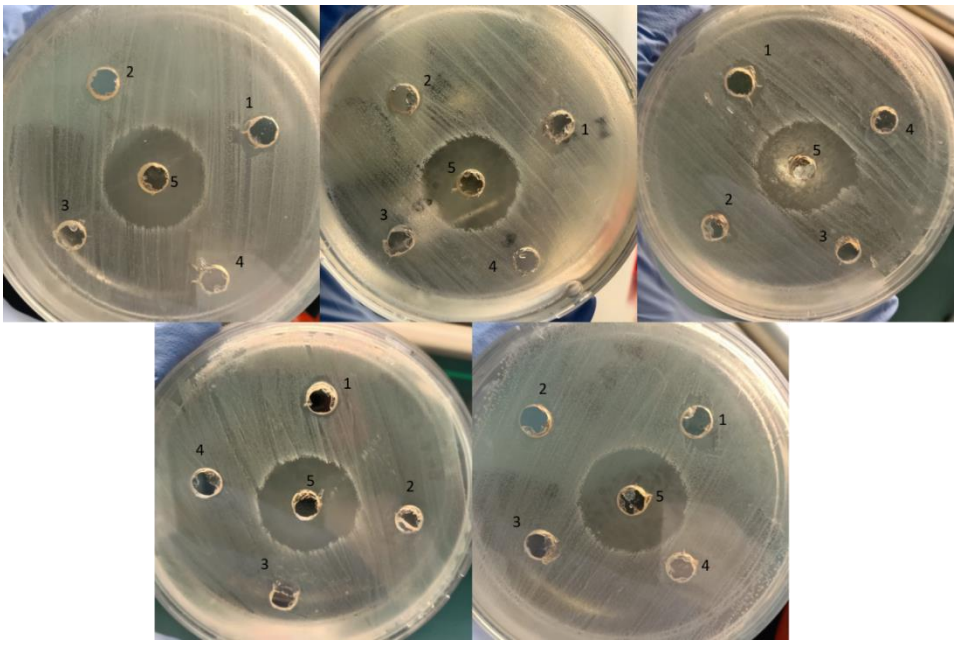

Figure 1. Antibacterial susceptibility test of GE results. The clear zone (inhibition zone) where EF bacteria were absent are the IZD

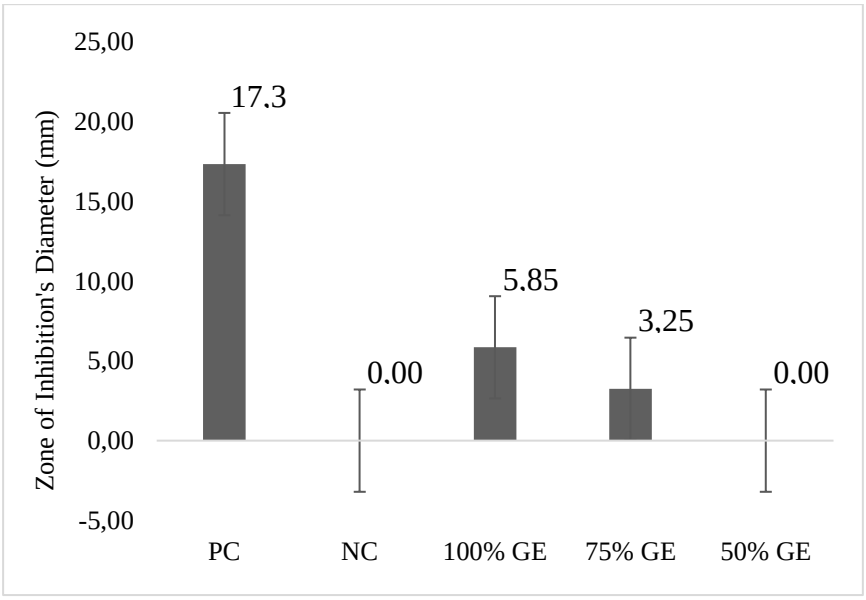

Figure 2. Antibacterial susceptibility test of GE results in mean difference ± standard deviation

Based on the results of the parametric test using One-way ANOVA, a significance value or $p < 0.05$ ($p = 0.000$) was obtained. Consequently, the null hypothesis (H_0) is rejected, and the alternative hypothesis (H_1) is accepted, indicating a significant difference between the IZD of GE treatments and the growth of EF. The parametric test results are presented in Table 2.

Table 2. Parametric test results using One-way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1028.441	4	257.110	2146.664	.000*
Within Groups	2.395	20	.120		
Total	1030.836	24			

* for significance value or $p < 0.05$

Based on the results of the follow-up test using Post-Hoc Dunnett T3, it can be concluded that there are significant differences in the ability of 100% GE, 75% GE, and PC to inhibit the growth of EF. The follow-up test results are presented in **Table 3**.

Table 3. Follow-up test results using Post-Hoc Dunnet T3 in mean difference ± standard deviation

	PC	NC	100% GE	75% GE	50% GE
PC		17,34 ± 0,26*	11,49 ± 0,32*	14,09 ± 0,28*	17,34 ± 0,26*
NC	-17,34 ± 0,26		-5,85 ± 0,19	-3,25 ± 0,11	0,00 ± 0,00
100% GE	-11,49 ± 0,32	5,85 ± 0,19*		2,60 ± 0,22*	5,85 ± 0,19*
75% GE	-14,09 ± 0,28	3,25 ± 0,11*	-2,6 ± 0,22		3,25 ± 0,11*
50% GE	-17,34 ± 0,26	0,00 ± 0,00	-5,85 ± 0,19	-3,25 ± 0,11	

* for significance value or $p < 0.05$

Discussion

GE has demonstrated antibacterial activity against EF, attributed to the presence of bioactive compounds with antimicrobial properties. Phytochemical analysis of GE revealed the presence of phenolic compounds, flavonoids, saponins, and steroids, which are likely contributors to its antibacterial effects. The study observed a correlation between GE concentration and IZD, indicating a concentration-dependent effect. The largest IZD of 5.85 mm was observed at the highest concentration (100%), while the smallest IZD of 3.25 mm was obtained with the 75% concentration. This suggests that as GE concentration increases, the abundance of bioactive compounds also increases, leading to a more pronounced antibacterial effect and a larger IZD.

Phenolics are one of the most important groups of natural antioxidants and are commonly found in plants either as free molecules or as esters/glycosides (Goleniowski et al., 2013). Phenolics exhibit broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria (Lobiuc et al., 2023). At high concentrations, phenolics can penetrate and disrupt the bacterial cell wall and precipitate proteins within bacterial cells, while at low concentrations, phenolics can inactivate essential enzyme systems within bacterial cells (Makarewicz et al., 2021). Phenolic compounds exert their antibacterial effects by damaging bacterial membranes and suppressing bacterial biofilm formation (Kauffmann & Castro, 2023).

Flavonoids are secondary metabolite compounds included in the group of phenol compounds whose benzene structure is substituted with the hydroxyl group (Liu et al., 2024). The mechanism of action of flavonoids as antibacterial compounds is by inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism (Hamzah & Shamsudin, 2020). In addition to inhibiting cell membrane function, flavonoids will form complex compounds from extracellular and dissolved proteins so that the cell membrane will be damaged and intracellular components will leak (Fitri & Suwondo, 2020). Flavonoids also inhibit energy metabolism by inhibiting the use of oxygen by bacteria, namely by preventing

the formation of energy in the cytoplasmic membrane and inhibiting bacterial motility (Ullah et al., 2020).

Saponins are compounds found in various plant species, both wild and cultivated (Zaynab et al., 2021). The mechanism of action of saponins as antibacterial agents involves protein denaturation (Mulia et al., 2023). Due to their detergent-like surface-active properties, saponins can lower the surface tension of the bacterial cell wall, thereby compromising membrane permeability (Tatli Cankaya & Somuncuoglu, 2021). This disruption of the cell membrane leads to leakage of intracellular contents, ultimately disrupting bacterial survival (Khameneh et al., 2021). Additionally, saponins can diffuse through the cytoplasmic membrane, further destabilizing it and causing leakage of cytoplasmic components, ultimately resulting in cell death (ALAMSJAH et al., 2024).

Steroids, a class of compounds with significant therapeutic applications, exhibit antibacterial properties by disrupting bacterial cell integrity and inducing leakage of intracellular contents. This mechanism involves increasing the permeability of the bacterial cell membrane, leading to the loss of essential cellular components (Crowley et al., 2022). While the study demonstrates the antibacterial efficacy of GE against EF bacteria, it falls short of matching the broad-spectrum antibacterial activity of CHX, particularly against Gram-positive bacteria (Aftab et al., 2022). CHX, at a concentration of 0.2%, acts as a bacteriostatic agent, primarily by inhibiting bacterial protein synthesis.

Garlic (*Allium sativum* L.) has established antibacterial properties, demonstrating efficacy against various bacterial strains. Previous studies have shown that GE effectively inhibits the growth of *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Mycobacterium tuberculosis* (Bhatwalkar et al., 2021). Additionally, GE exhibited antibacterial activity against oral bacteria such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Streptococcus mutans*. These findings corroborate previous research, further supporting the antibacterial efficacy of GE.

CONCLUSION

The effect of GE in inhibiting the growth of EF was observed by the presence of inhibition zones at 100% and 75% concentrations, although it was not yet able to match the effectiveness of 0.2% CHX.

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