Evaluation of the Antibacterial Efficacy of Allium Sativum Gel as Intracanal Medicament Versus Calcium Hydroxide Paste Against Enterococcus faecalis in Single Rooted Teeth

(A Comparative In-Vitro Study)

تقييم الفاعلية المضاده للبكتيريا لعلاصه نبات الثوم كدواء داخل القناة مقابل معجون هيدروكسيد الكالسيوم ضد بكتيريا المكورة المعوية البرازية في الأسنان أحادية الجذور

(دراسه مقارنة معملية)

Protocol submitted to

Faculty of Dentistry, Cairo University

For partial fulfillment of the requirements for master's degree in Endodontics.

By:

Rania Ahmed Ahmed Elnaa

B.D.S (2017)

Faculty of Dentistry

Tanta University

1ry signature of research plan committee

Prof Dr Ghada ElHitaly Eid

Dr Ghada ElHitaly Eid 22/5/2024

Dr Ahmed Shawky El Sheshtawy

Code:New
ENDO 3-6-1

Head of department's signature

Supervisors' Signature

1- Prof. Lubna Shafie

2-

3-

Endodontic Research plan Serial No
May 2024 -ERP code No.13: Protocol (1)

Protocol Checklist				
Section and topic	**Item no.**	**Checked item**	**Reported on page No.**	**Reviewer's check**
I. Administrative information	1	Title	1	
	2	Protocol registration	1	
	3	Protocol version	1	
	4	Funding	1	
	5	Roles and responsibilities	1,2	
II. Introduction				
	6a	Scientific background	3,4	
	6b	Specific objectives	5,6,7,8	
III. Methods				
A) Samples, intervention and outcomes	7	Calculated sample size	9	
	8	Description of samples	9,10,11	
	9	The intervention for each group	11,12	
	10	Outcomes	12,13	
B) Assignment to intervention		If randomized report the following:		
	11	Sequence generation	14	
	12	Allocation concealment mechanism	14	
	13	Implementation	14	
C) Blinding	14	Blinding	14	
D) Statistical methods	15	Statistical methods for comparisons of groups	14	
IV- References	15,16,17,18			

Evidence based committee (Reviewers)		
Name	**Signature**	**Date**
1.		
2.		
Research plan committee		
Name	**Signature**	**Date**
1.		
2.		
Ethics	14	

I. Administrative information

1. Title:

Evaluation of the Antibacterial Efficacy of Allium Sativum Paste as Intracanal Medicament Versus Calcium Hydroxide Paste Against Enterococcus Faecalis in Single Rooted Teeth (A Comparative In-Vitro Study)

2. Protocol Registration:

This protocol will be registered on www.clinicaltrials.gov

3. Protocol version:

Date: April 2024

4. Funding: Self-funded

5. Roles and responsibilities:

A. Rania Ahmed Ahmed Elnaa

- **Affiliation:** Master's degree student at the Department of Endodontics - Faculty of Dentistry Cairo University.
- **Role:** Main researcher.
- **Responsibilities**: Collecting and entering the baseline data required in the study, Collect the appropriate teeth for the study, Methods performer, writing the thesis, interpretation of results, and drawing out conclusion.
- **Email: Rania.elnaa@dentistry.cu.edu.eg**

B. Prof. Dr. Lubna Abbas Shafie (Main Supervisor)

- **Affiliation:** Professor at the Department of Endodontics - Faculty of Dentistry – Cairo University.
- **Role:** chief supervisor.
- **Responsibilities**: Monitoring and editing the data and the whole process of the study, Accesses the results and data collected from the study.
- **Email: Dr.lubna.shafie@gmail.com**

C. Dr. Shaimaa Ibrahim Bakry

- **Affiliation:** Lecturer at the Department of Endodontics – Faculty of Dentistry-Cairo University.
- **Role:** Co-supervisor.
- **Responsibilities:** Generates a random sequence of the samples, and undergoes proper allocation concealment, implementation and access to the final data, monitor the process of data collection.
- **Email: Shaimaa.Bakry@dentistry.cu.edu.eg**

D. Prof. Dr. Laila Ahmed Rashed

- **Affiliation:** Professor of Medical Biochemistry and Molecular Biology - Faculty of Medicine - Cairo university.
- **Role:** Co-supervisor.
- **Responsibilities:** Monitoring and supervising the isolation, culturing and quantification of bacteria before and after the disinfection protocol.
- **Email: Lailarashed@kasralainy.edu.eg**

II. Introduction

A. Scientific background

Statement of the problem

Two fundamental goals of endodontic treatment are to prevent or treat apical periodontitis. From a predictive perspective, several variables can affect the outcome of root canal treatment. Some of these variables depend on intraoperative factors, which include irrigation technique, size of the apical preparation, use of intracanal medicaments or the number of appointments necessary to complete the treatment. However, the outcome may also be affected by host and microbial factors.(Ordinola-Zapata et al., 2022)

Unfortunately, not all cases offer a favorable prognosis. The presence of a long-standing infection, or an extra-radicular infection and the inability to reach microorganisms in inaccessible areas (i.e., apical ramifications) are cases present in dental practice with a poor or questionable prognosis. In these cases, the use of an intracanal medicament after thorough debridement can improve the disinfection of the root canals and improving its success.(Ordinola-Zapata et al., 2022)
E. faecalis are anaerobic facultative gram-positive bacteria with powerful virulence factors including the ability to compete with other microorganisms, invade the dentinal tubules, and survive nutritional deficiencies. Adhering to the root canal walls, accumulating to form biofilms, and so becoming more resistant to irrigation substances and intracanal medicaments, they are consequently often associated with persistent infections and root canal treatment failures.(Stuart et al., 2006)

Calcium Hydroxide Ca $(OH)_2$ is the most widely used intracanal medicament in necrotic cases reducing the signs and symptoms. It possesses many advantageous properties such as high alkalinity, biocompatibility and antibacterial effect. Resistance to calcium hydroxide has been found in some microbial species such as *Enterococcus faecalis* and *Candida albicans*. *E. faecalis* and *C. Albicans* are commonly found in cases with persistent or extra-radicular infections.(Kim & Kim, 2014)

Calcium hydroxide could induce monocytes and trigger the release of tumor necrosis factor α (TNFα) and prostaglandin E2 (PGE2), which are responsible for periapical tissue destruction. For this reason, judging the contact time required for the intracanal medication inside the root canal lumen to eradicate the residual pathogen is important. There should be a balance between achieving proper antibacterial action with minimal biological and mechanical side effects.(A. A. Khan et al., 2008)

In recent years, a predominant interest has been observed in evaluating different plant extracts for their antimicrobial properties against bacteria causing dental caries and periradicular pathology. The constant increase in antibiotic-resistant strains and side effects caused by synthetic drugs has prompted the researchers to look for herbal alternatives in endodontics. (Ambareen & Chinappa, 2014)

Amongst these antibacterial herbal agents, *Allium sativum* (AS) is readily available worldwide, economic and shows superior antibacterial property.(Chen et al., 2020)

The main active component of garlic is allicin. It is antibacterial and has immune regulatory functions. Allicin destroys cell wall and cell membrane of root canal bacteria. It was used as an alternative irrigant to Sodium hypochlorite (NaOCL).(Magryś et al., 2021)

Previous studies used *Allium sativum* as an irrigant and showed promising clinical results, because A. Sativum has a therapeutic effect via its broad-spectrum antibacterial effect as well as its less cytotoxic effect. (Fani et al., 2007; L. Khan et al., 2014; Perez-Giraldo et al., 2003)

However, according to our current knowledge, it is not clear whether the Allium Sativum extracts' usage as an intracanal medicament could give more antibacterial action than the traditionally used calcium hydroxide or not. So, the aim of this study is to evaluate the antimicrobial efficacy of garlic extract in comparison to calcium hydroxide paste against *Enterococcus faecalis* developed in single rooted root canals.

B- Review of literature

In 2008, Sawsan T. Abu Zied et al. evaluated antibacterial activities of two natural plants (freshly minced garlic extract and fresh lemon solution) against three traditional intracanal medications (10% citric acid , 5.25%NaOCl, and Camphorated Para Chloro-Phenol(CPCP)) on mixed root canal flora) and four bacterial strains (α-hemolytic Streptococci, Streptococci pyrogens, Enterococci faecalis and Pseudomonas aeruginosa). The results showed that both freshly minced garlic and CPCP represented the statistically significant largest inhibitory zones when compared with other three medications used. The vials containing samples collected from root canal medicated with CPCP appeared the clearest media, followed by that medicated with minced garlic followed by NaOCl and fresh lemon solution and citric acid respectively. It was concluded that fresh minced garlic and fresh lemon solution may be useful as intracanal medications, as they inhibited the growth of all bacteria used.

In 2013, Eswar et al. evaluated the efficacy of garlic extract with 2% chlorhexidine (CHX) and calcium hydroxide Ca $(OH)_2$ in disinfection of dentinal tubules contaminated with Enterococcus faecalis by using real-time polymerase chain reaction (PCR). They concluded that 2% CHX showed better antibacterial efficacy against E. faecalis by using real- time PCR while Garlic extract showed better antibacterial efficacy compared to Ca (OH)2 against E. faecalis.

In 2015, Ourvind J. et al. evaluated the anti-microbial efficacy of garlic extract (GE) against Enterococcus faecalis biofilm and its ability to penetrate into root dentin. E. faecalis was cultured and treated with the test agents – normal saline, 5.25% of NaOCl, and the three different concentrations of GE (10%, 40%, and 70%). The experiment was done in four groups namely, 24-h Co-treatment group, 24-h biofilm treatment group, 1-week biofilm group, and 3-week biofilm group. These groups were subjected to microbial viability assay and fluorescence microscopic analysis. The most effective concentration of garlic (70%) was further tested and compared with 5.25% NaOCl. The results revealed that GE was able to disrupt as well as prevent the formation of biofilm produced by E. faecalis and exhibited similar anti-microbial efficacy as 5.25% NaOCl. It was concluded that, GE has a potential to serve as an alternative herbal root canal irrigant.

In 2015, Swati Ramesh Karkare et al. evaluated the antimicrobial activity of saturated and diluted (1:1) hydroalcoholic extract of Aloe vera, garlic compared to 5% NaOCl against E. faecalis by the method of agar diffusion. The results showed that saturated hydroalcoholic extract of A. vera had the highest zone of inhibition against E. faecalis, and concluded that saturated hydroalcoholic extract of A. vera, garlic could replace NaOCl, which was considered as gold standard irrigant.

In 2019, ANDRIANI OCTAVIA et al. evaluated the antibacterial effect exhibited by garlic extract against some anaerobic bacteria such as Lactobacillus and E. faecalis. They used (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) MTT assay to determine the viability of E. faecalis after exposure to increasing concentrations of garlic extract (10%, 25%, 50%, and 100%) and chlorhexidine (CHX) 2% as positive control. The results showed that at all concentrations of garlic extracts decreased the viability of E. faecalis.

In 2022, Shaimaa M. Omer et al. evaluated the antibacterial effects of *Allium sativum* (garlic extract) and calcium hydroxide (Ca $[OH]_2$) as intracanal medicaments in infected mature anterior teeth using real-time PCR. The study was carried out on 66 permanent, necrotic incisors were randomly divided into three groups ($n = 22$) in each group. After access preparation, four microbiological samples (S) were taken using sterile absorbent paper points as follows, S1: before canal instrumentation and S2: after cleaning and shaping. The third sample (S3) and fourth sample (S4) were taken after the placement of the tested intracanal medications into canals for 7 and 14 days, respectively. The results showed that garlic significantly reduced *Enterococcus faecalis* FC in S3 and S4 when compared to Ca $(OH)_2$.

In 2023, Khushi Chetankumar Patel et al. evaluated the antimicrobial efficacy of (neem, garlic extract and Tridax procumbens) and sodium hypochlorite against E- faecalis. It was measured using agar well diffusion. The results showed that sodium hypochlorite indicated the maximum inhibitory zone against E. faecalis, subsequently neem, garlic extract and the lowest by Tridax procumbens. They concluded that Neem, garlic extract, and Tridax irrigants are suitable for use as root canal irrigating solutions.

b. Objectives

The aim of this study is to evaluate the antimicrobial efficiency of garlic extract as an intracanal medicament in comparison to calcium hydroxide paste against *Enterococcus faecalis* viability developed in single rooted root canals by bacterial DNA extraction method using real time PCR.

c. Research Question

Will application of intracanal medicament of garlic extract differ from calcium hydroxide paste in reduction of *E. Faecalis* count when used in single rooted teeth?

d. Null Hypothesis

There is no difference between the antimicrobial efficacy of garlic extract and calcium hydroxide paste in reduction of *E. faecalis* count in single rooted teeth.

e. PICO

P: Population

Single rooted human freshly extracted teeth with single canals.

I: Intervention

Garlic extract used as intracanal medication.

C: Control

Calcium hydroxide paste used as intracanal medication.

O: Outcome

Bacterial load reduction determined by using real time PCR technique after root canal preparation.

Outcome	Method of Measurement	Unit of Measurement	Time
• Bacterial load reduction.	Quantitative Real time PCR.	Percentage	Sample 1 (S1): after inoculation of root canals& before instrumentation. Sample 2 (S2): after instrumentation. Sample 3 (S3): after intracanal medicament placement for 1 week. Sample 4 (S4): after intracanal medicament placement for 2 weeks.

III. Materials and Methods

1) Samples:

- **Calculated Sample Size:**

In the study by **Kalaiselvam et al (2019)**, the mean E. faecalis bacterial load in the calcium hydroxide group was found to be 0.8 SD (0.2). Assuming a Cohen's d effect size of 1.5, a type I error of 0.05 and a study power of 0.8, the sample required to detect the minimum significant difference between the two groups was calculated and found to be 10 samples per group with a total of 40 samples.

The sample size was calculated using G Power software version 3.1.9.7

- **Sample description:**

Human permanent single rooted teeth extracted due to orthodontic, periodontal or prosthodontic reasons will be collected from the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Cairo University.

- **Eligibility criteria:**

Inclusion criteria:

- Human permanent single rooted teeth with single canal.
- Mature root apices.

Exclusion criteria:

- Teeth with root curvature.
- Teeth with root caries, root irregularities or other anomalies.
- Teeth with calcified root canals.
- Teeth with internal or external root resorption.
- Cracked teeth.
- Teeth with previous endodontic manipulation.

• **Sample Preparation &canal inoculation:**

1. Any calculus or soft tissue remnants will be removed by using ultrasonic scaler [i,ii] .

2. Crowns of all teeth will be cut off at cemento-enamel junction using a high-speed hand piece and the root lengths will be standardized to a (15 ±1 mm) length.

3. All the samples will be sterilized at 121°C for 30 minutes, one tooth will be randomly selected and incubated to ensure the negative culture and sterilization of samples.

4. Standard strain of Enterococcus faecalis (ATCC 29212) will be used and revived on blood agar at 35°C for 48 h. Inoculum will be prepared in sterile brain heart infusion broth (BHI) and turbidity will be adjusted to 0.5 McFarland corresponding to approximately (1.5×10^8) colony forming units per milliliter (CFU/mL).(Abd El-Majeed et al., 2020)

5. The apical foramen will be sealed using a resin composite and the external surfaces of the teeth will be covered with two layers of colorless nail varnish to prevent liquid seepage then 5 ml of bacteria will be placed into each root canal, thereafter, each sample will be placed in a sterile Eppendorf tube containing BHI and will be stored at 37°C for 2 weeks.

6. During this period, BHI will be refreshed every 3 days.

7. The excess broth will be eliminated from the root canal with paper points at the end of incubation period and the first microbial sample (S1) will be taken and stored in DNA lysis solution and immediately placed in an ice box containing ice or directly into a freezer (-80℃) for further assessment.

(i)Woodpecker UDS-K led ultrasonic scaler, Guilin Woodpecker Medical Co., Ltd., Guangxi, China.
(ii)NSK ultrasonic scaler Various 570, NAKANISHI INC., Tochigi, Japan.

- **Root canal preparation& medicament application:**

1. Patency will be established using a #15 hand file, the working length (WL) will be established 1 mm shorter than the apical foramen.

2. All the root canals will be prepared with rotary files till size #35/04 [iii] according to the manufacturer's specifications.

3. After each instrument change, canals will be passively irrigated with 5 ml of saline solution using 30 Gauge side vented needle for 1 min then (S2) is taken and stored as mentioned in (S1).

• **Medication preparation &insertion:**

4. The samples will be divided randomly into 2 groups:

5. **G1 (garlic extract gel)** Garlic (80 g) will be put in a large bowl, pounded finely, and added to a 600 ml beaker. Absolute ethanol (160 ml) will be added and left at room temperature for 60 minutes. Then the solution will be filtered with paper No. 4 into a 500 ml round bottom flask. The garlic extract will be concentrated with a vacuum evaporator at room temperature and low pressure until no more solvent evaporates. After filtering the garlic juice, the garlic residue will be left on the filter paper. Then, the garlic residue will be dried and weighed equal to 5 mg of garlic extract.

Hydrogel will be prepared using 0.3% (30mg) of Carbopol Ultrez. Carpobol Ultrez and 10 ml of distilled water will be added to the beaker and mixed. Then garlic extract will be added. Neutralization and cross-linking will be achieved by adding 0.5 ml of triethanolamine. Then propyl paraben will be added as a preservative.(ÇAĞLAR et al., 2023)

G1A garlic extract gel will be applied into the canals for 7 days.

G1B garlic extract gel will be applied into the canals for 14 days.

G2 (Calcium hydroxide paste) positive control metapaste [vi].

G2A calcium hydroxide paste will be applied into the canals for 7 days.

G2B calcium hydroxide paste will be applied into the canals for 14 days.

(iii) Dota S-One, Dota Endo, China.

(vi) Meta Biomed MetaPaste Calcium Hydroxide Paste With Barium Sulphate.

6. The teeth in both groups will be sealed temporarily and incubated at 37°C for 7days.
7. After this period, each tooth will be irrigated with 5 ml saline to remove intra canal medication from the canal then S3 will be collected and stored as mentioned before.
8. After 14 days, sample 4 (S4) will be taken after placement of the intracanal medications for 14 days and irrigation of the canals with 5 ml saline to remove intracanal medications from the canals.

2) Outcome assessment

It will be determined by using the real-time polymerase chain reaction (PCR) method in Biochemistry Department- Cairo University.

The first sample will be taken from each canal after bacterial inoculation before implementing any of the procedures. The canals will be filled sterile saline using 1 ml insulin syringe. A new sterile #20 H-file will be used to gently scrap the canal walls to disturb the contents of the canal, then the content will be collected by using several paper points. In a test tube in which there was 1 ml of saline solution, paper points will be soaked and shake for 30 seconds and stored in DNA lysis solution and immediately placed in an ice box containing ice or directly into a freezer (-80℃) until further investigations. The second, third and fourth samples will be tested the same as the first.

Outcome	Assessment method	Unit	Time
Bacterial load reduction	Quantitative Real time PCR	Percentage	Sample 1 (S1): after inoculation of root canals & before instrumentation. Sample 2 (S2): after instrumentation. Sample 3 (S3): after intracanal medicament placement for 1 week. Sample 4(S4): after intracanal medicament placement for 2 weeks.

4-Assignment to intervention

Sequence generation

Random allocation and sequence generation will be performed using computer random sequence generator program (https//:www.random.org).

Allocation concealment

To prevent the selection bias in the interventions, the allocated sequence will be protected and concealed until assignment using sequentially numbered opaque sealed envelopes in which the teeth will be placed.

Implementation

Random allocation, sequence generation and the allocation concealment will be performed by the Co-supervisor.

Blinding

The outcome assessor and the statistician will be blinded to the intervention.

D)Statistical methods

Data will be examined for normality using histograms and Shapiro Wilk test. Continuous data will be presented as mean, standard deviation, median and range values. ANOVA test will be used for between-group comparison of parametric continuous data from non-related samples followed by Tukey's post hoc test for pairwise comparisons. Kruskal Wallis test will be used for between group comparison of non-parametric continuous data from non-related samples followed by Dunn's post hoc test for pairwise comparisons. The level of significance will be set for all tests at $p = 0.05$.

IV- Ethics:

The research will be admitted to the ethics committee for reviewing**.** After receiving the results and finishing the experiment, all the instruments and teeth samples will be sterilized and discarded in a special incinerator under supervision of Biochemistry department- Cairo University

IV-References

Abd El-Majeed, S., Nour El-deen, M., Rokaya, M., & Sherif, M. (2020). Comparative Evaluation of the Antibacterial Effect of Triple Antibiotic Paste Vesus Propolis as Intracanal Medicament. *Al-Azhar Dental Journal for Girls*, *7*(1), 1–6. https://doi.org/10.21608/adjg.2019.6578.1063.

Ambareen, Z., & Chinappa, A. (2014). Go Green- Keep the Root Canal Clean!!! *International Journal of Dental Sciences and Research*, *2*(6B), 21–25. https://doi.org/10.12691/ijdsr-2-6B-7.

Birring, OurvindJ. S., Viloria, I., & Nunez, P. (2015). Anti-microbial efficacy of Allium sativum extract against Enterococcus faecalis biofilm and its penetration into the root dentin: An in vitro study. *Indian Journal of Dental Research*, *26*(5), 477. https://doi.org/10.4103/0970-9290.172041.

ÇAĞLAR, E. Ş., KARAOTMARLI GÜVEN, G., & ÜSTÜNDAĞ OKUR, N. (2023). PREPARATION AND CHARACTERIZATION OF CARBOPOL BASED HYDROGELS CONTAINING DEXPANTHENOL. *Ankara Universitesi Eczacilik Fakultesi Dergisi*, *47*(3), 6–6. https://doi.org/10.33483/jfpau.1195397.

Chen, Y.-Y., Chiu, H.-C., & Wang, Y.-B. (2020). Effects of garlic extract on acid production and growth of Streptococcus mutans. *Journal of Food and Drug Analysis*, *17*(1). https://doi.org/10.38212/2224-6614.2309.

Daga, P., Asrani, H., Farista, S., & Mishra, P. (2017). Comparative Evaluation of Antimicrobial Efficacy of Neem, Miswak, Propolis, and Sodium Hypochlorite against Enterococcus faecalis using EndoVac. *International Journal of Prosthodontics and Restorative Dentistry*, *7*(2), 60–65. https://doi.org/10.5005/jp-journals-10019-1178.

Eswar, K., Venkateshbabu, N., Rajeswari, K., & Kandaswamy, D. (2013). Dentinal tubule disinfection with 2% chlorhexidine, garlic extract, and calcium hydroxide against Enterococcus faecalis by using real-time polymerase chain reaction: In vitro study. *Journal of Conservative Dentistry*, *16*(3), 194. https://doi.org/10.4103/0972-0707.111312.

Fani, M., Kohanteb, J., & Dayaghi, M. (2007). Inhibitory activity of garlic (*Allium sativum*) extract on multidrug-resistant *Streptococcus mutans*. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, *25*(4), 164. https://doi.org/10.4103/0970-4388.37011.

Kalaiselvam, R., Soundararajan, K., Rajan R, M., Deivanayagam, K., Arumugam, C., & Ganesh, A. (2019). Comparative Evaluation of the Anti-bacterial Efficacy of Herbal Medicaments and Synthetic Medicaments Against Enterococcus faecalis using Real-time Polymerase Chain Reaction. *Cureus*. https://doi.org/10.7759/cureus.5228.

Kandaswamy, D., Venkateshbabu, N., Gogulnath, D., & Kindo, A. J. (2010). Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide. *International Endodontic Journal*, *43*(5), 419–423. https://doi.org/10.1111/j.1365-2591.2010.01696.x.

Karkare, S., Ahire, N., & Khedkar, S. (2015). Comparative evaluation of antimicrobial activity of hydroalcoholic extract of Aloe vera, garlic, and 5% sodium hypochlorite as root canal irrigants against Enterococcus faecalis: An in vitro study. *Journal of Indian Society of Pedodontics and Preventive Dentistry*, *33*(4), 274. https://doi.org/10.4103/0970-4388.165658.

Khan, A. A., Sun, X., & Hargreaves, K. M. (2008). Effect of Calcium Hydroxide on Proinflammatory Cytokines and Neuropeptides. *Journal of Endodontics*, *34*(11), 1360–1363. https://doi.org/10.1016/j.joen.2008.08.020.

Khan, L., Paulino, E. M., Lim, D., Nadela, F., Yadav, R., & Birring, O. S. (2014). Anti-microbial efficacy of Allium sativum against Streptococcus mutans biofilm formation on orthodontic mini-implants. *Journal of Orthodontic Research*, *2*(3), 129. https://doi.org/10.4103/2321-3825.140683.

Kim, D., & Kim, E. (2014). Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part I. *In vitro* studies. *Restorative Dentistry & Endodontics*, *39*(4), 241. https://doi.org/10.5395/rde.2014.39.4.241.

Magryś, A., Olender, A., & Tchórzewska, D. (2021). Antibacterial properties of Allium sativum L. against the most emerging multidrug-resistant bacteria and its synergy with antibiotics. Archives of Microbiology, 203(5), 2257–2268. https://doi.org/10.1007/s00203-021-02248-z.

Mahfouz Omer, S. M., Mohamed, D. A., & Ali Abdel Latif, R. M. (2022). Comparative evaluation of the antibacterial effect of *Allium sativum* , calcium hydroxide and their combination as intracanal medicaments in infected mature anterior teeth: A randomized clinical trial. *International Endodontic Journal*, *55*(10), 1010–1025. https://doi.org/10.1111/iej.13801.

OCTAVIA, A., BUDIARDJO, S. B., INDIARTI, I. S., FAUZIAH, E., SUHARSINI, M., SUTADI, H., & RIZAL, M. F. (2019). GARLIC EXTRACT EFFICACY AGAINST THE VIABILITY OF ENTEROCOCCUS FAECALIS (IN VITRO). *International Journal of Applied Pharmaceutics*, 194–197. https://doi.org/10.22159/ijap.2019.v11s1.17351.

Ordinola-Zapata, R., Noblett, W. C., Perez-Ron, A., Ye, Z., & Vera, J. (2022). Present status and future directions of intracanal medicaments. *International Endodontic Journal*, *55*(S3), 613–636. https://doi.org/10.1111/iej.13731.

Perez-Giraldo, C., Cruz-Villalon, G., Sanchez-Silos, R., Martinez-Rubio, R., Blanco, M. T., & Gomez-Garcia, A. C. (2003). In vitro activity of allicin against Staphylococcus epidermidis and influence of subinhibitory concentrations on biofilm formation. *Journal of Applied Microbiology*, *95*(4), 709–711. https://doi.org/10.1046/j.1365-2672.2003.02030.x.

STUART, C., SCHWARTZ, S., BEESON, T., & OWATZ, C. (2006). Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment. *Journal of Endodontics*, *32*(2), 93–98. https://doi.org/10.1016/j.joen.2005.10.049.

Zied, S. T. A., & Eissa, S. A. L. (n.d.). *Comparative Study On Antibacterial Activities Of Two Natural Plants Versus Three different intracanal Medications*.