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Inhibition of Citronella Essential Oil (*Cymbopogon Nardus*) Bengkulu on Bacterial Growth after Endodontic Treatment

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ABSTRACT

Background: Root canal treatment aims to address pulpal infection and prevent reinfection, which is often caused by bacteria such as *Enterococcus faecalis* and *Staphylococcus aureus*. Citronella essential oil (*Cymbopogon nardus*) from Bengkulu, containing antibacterial compounds, has potential to inhibit the growth of these bacteria.

Objective: This study evaluated the effectiveness of citronella essential oil against *E. faecalis* and *S. aureus*.

Materials and Methods: The method used was the disc diffusion technique with 20 samples each of *E. faecalis* and *S. aureus* in BHI media. Citronella essential oil was tested at concentrations of 50%, 75%, and 100%, with 2% CHX as the positive control. The inhibition zones were measured after 24 hours.

Results: The highest inhibition for *E. faecalis* was found at 100% concentration (2.56 mm), while for *S. aureus*, it was at 50% concentration (1.67 mm). Significant differences ($p<0.05$) were observed at all concentrations of citronella essential oil for both bacteria.

Conclusion: Citronella essential oil from Bengkulu exhibited weak antibacterial activity against *E. faecalis* and *S. aureus*. Its potential as an alternative for root canal treatment requires further development.

KEYWORDS: Pulpal and periapical diseases, Bacteria in the root canal, Root Canal Treatment, Root canal medicaments, 2% Chlorhexidine Gluconate, Citronella essential oil.

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1. INTRODUCTION

The Basic Health Research by the Ministry of Health in 2018 revealed that the main dental and oral health problems in Indonesia are damaged and decayed teeth, as well as toothache, affecting 45.3% of the population.[1] The prevalence of dental caries in Indonesia reaches 88.8%, with root caries prevalence at 56.6%.[2]

Dental caries is the most commonly encountered clinical issue that leads to pulp and periapical diseases.[3] The pulp consists of connective tissue that includes nerves, blood vessels, ground substance, intercellular fluid, odontoblasts, fibroblasts, and other cellular elements. Additionally, the dental pulp contains vascular connective tissue within the dentin walls.[4]

Pulp and periapical diseases can be treated with curative care, primarily through root canal therapy (endodontics). This procedure involves removing infected pulp tissue from the pulp chamber and root canals, followed by filling these spaces with special materials to prevent future infection.[5,6] The main goal of root canal therapy is to eliminate bacteria from the root canals and prevent reinfection, allowing the tooth to survive and function in the oral cavity for as long as possible. One crucial step in this process is the use of root canal medication, which aims to sterilize pathogenic microorganisms within the root canals. These medications are vital for addressing any remaining bacteria after preparation.[7,8]

One of the main causes of failure in endodontic treatment is microbial infection, which can occur inside the root canal or in the surrounding area. Most of the bacteria present in the root canal are anaerobic, with 90% of them being anaerobic species. Anaerobic bacteria that often cause

treatment failure include *Staphylococcus aureus* and *Enterococcus faecalis*.[8]

Root canal medications are generally antimicrobial agents aimed at thoroughly eliminating bacteria between treatment sessions and reducing the risk of aseptic complications.[9] However, these medications often have limitations, such as high toxicity, resistance to certain bacteria, potential sensitization, and allergic reactions. One medication proven effective in inhibiting anaerobic bacteria is 2% Chlorhexidine Gluconate, which is commonly used in root canal procedures.[4]

Although effective, 2% Chlorhexidine has limitations, including its inability to dissolve necrotic tissue, reduced effectiveness against gram-negative bacteria, and no impact on biofilm. Therefore, it is necessary to explore herbal-based root canal medications that are more compatible with tissues while still offering antibacterial properties comparable to non-herbal alternatives.[3,11]

Citronella (*Cymbopogon nardus*) is a medicinal plant with many benefits and originates from tropical Asia. This plant is widely cultivated across Indonesia, including in Bengkulu. Citronella is rich in compounds such as flavonoids, alkaloids, saponins, tannins, anthraquinones, and essential oils, which have antibacterial, anti-inflammatory, antifungal, and antioxidant effects.[12,13]

The essential oil in citronella (*Cymbopogon nardus*) accounts for approximately 0.7% of its total composition. This oil is obtained through the distillation of the leaves and stems of citronella. Citronella essential oil shows antibacterial, antifungal, and antioxidant properties, primarily due to its main components: geraniol, citronellal, and citronellol.[14]

Research by Sari et al. in 2022 found that citronella essential oil effectively inhibits MRSA, with the largest inhibition zone at a 10% concentration.[15] Conversely, a study by Howarto et al. in 2015 reported that citronella oil exhibited antibacterial activity against *Enterococcus faecalis* at a 100% concentration, though its effectiveness was relatively low compared to the positive control, clindamycin.[8] A study by Tanjung et al. in 2022 showed that lemongrass extract (*Cymbopogon citratus*) was effective against *Streptococcus mutans* at concentrations of 20%, 30%, 40%, and 50%.[16] However, research by Fitriani in 2021 revealed that citronella extract (*Cymbopogon nardus*) at concentrations of 10% to 100% was not effective in inhibiting the growth of *Escherichia coli* ATCC 25922.[1]

2. MATERIAL AND METHODS

2.1 Research type and design

This type of research is an experimental laboratory study using a posttest-only control group design.

2.2 Research materials (Sampels)

The research sample tested in this study was citronella essential oil (*Cymbopogon nardus*) which grow in Bengkulu with concentrations of 100%, 75%, and 50%. The positive control used CHX 2% solution (ONEMED®). The total number of samples used was 20 of *Enterococcus faecalis* and *Staphylococcus aureus*.

2.3 Sterillization of research tools

The tools used in the study were sterilized beforehand. They were thoroughly cleaned, dried, and wrapped in aluminum foil. Next, the tools were sterilized using an autoclave at a temperature of 121°C for 15 minutes. Other tools, such as inoculating needles, were sterilized by passing them over a spirit lamp flame.[29]

2.4 Preparation of citronella essential oil extract solution

The procedure for making extract solutions and testing the inhibitory power of citronella essential oils was carried out at the MICORE Laboratory of the Trisakti Faculty of Dentistry, Jakarta. Citronella essential oil is produced from lemongrass through a steam distillation process carried out directly in Bengkulu. The essential oil is prepared in several specific concentrations by mixing 100% citronella essential oil with 5% DMSO and 1% Tween 20, resulting in concentrations of 50%, 75%, and 100% citronella essential oil.[31] The concentration of 100% essential oil is made by dissolving 2 ml of citronella essential oil into 2 ml of DMSO 5% + 1% of *tween* 20. The concentration of 75% essential oil concentration was made by dissolving 1.5 ml of citronella essential oil into 1 ml of DMSO 5% + 1% of *tween* 20. The concentration of 50% extract concentration was prepared by dissolving 0,75 ml of citronella essential oil into 1 ml of DMSO 5% + 1% of *tween* 20.

2.5 Preparation of BHI Agar media

The preparation of Brain Heart Infusion Agar (BHI-A) media is done by weighing 52 grams and dissolving it in 1000 ml of pure water/distilled water. Heat until boiling to ensure the media is completely dissolved. After the media is dissolved, it must be sterilized using an autoclave at a pressure of 15 lbs (121°C) for 15 minutes. The media is then removed and cooled to 45-50°C. Stir well and pour into sterile petri dishes.

2.6 Prepaaration of BHI Broth media

In the preparation of Brain Heart Infusion Broth (BHI-B) media, 37 grams of powder is added to 1000 ml of pure water/distilled water. Heat to ensure the media is completely dissolved. Pour into a bottle or tube. Sterilize using an autoclave at a pressure of 15 lbs (121°C) for 15 minutes.

2.7 Preparation of *Enterococcus faecalis* and *Staphylococcus aureus* suspension

Preparation of *Enterococcus faecalis* and *Staphylococcus aureus* bacterial suspension was carried out by taking 1 ose of culture and then inserted in a tube containing 2 ml of Brain Heart Infusion Broth (BHI-B) then homogenized and incubated for 24 hours at 37o aerobically. The turbidity of the bacterial suspension was measured with a microplate reader with the Mc Farland 0.5 standard (1.5x108 CFU/ml).

2.8 Inhibition activity test of *Enterococcus faecalis* and *Staphylococcus aureus*

The research was conducted using the Kirby-Bauer method (disk diffusion) by preparing a bacterial culture medium using BHI-A, followed by culturing the bacteria. Next, a bacterial suspension was prepared by taking one inoculating loop from the culture and placing it in a tube containing 2 ml of Brain Heart Infusion Broth (BHI-B), which was then homogenized and incubated for 24 hours at 37°C under anaerobic conditions. The bacterial suspension was taken using a sterile loop and spread thoroughly over the BHI-A, repeating this process twice and rotating the dish by 60° to ensure even distribution of the bacteria.

Subsequently, the essential oil variables of citronella at 50% and 75% concentrations were prepared by diluting them with 1% Tween 20 and 5% DMSO. The filter paper discs were dipped into each stock of citronella essential oil at 50%, 75%, 100%, and the control K+ CHX 2% using sterile tweezers. The discs that had been dipped in the extracts and control substances were placed on the surface of the BHI-A medium that had been inoculated with the bacterial suspension, and then incubated at 37°C for 24 hours under anaerobic conditions. The method for measuring the inhibition zone involves turning the petri dish upside down and then measuring it using calipers, with the measurements recorded. The diameter of the inhibition zone that forms indicates the antibacterial effect of citronella essential oil.

Data Analysis

The data of the study were analyzed using IBM SPSS 29 *software*. The normality test of the data of the study results was carried out using *Shapiro-Wilk. One-way* ANOVA test to see significant differences in all groups. *Bonferroni Post Hoc* test to see significant differences between pairs of treatment groups studied

3. RESULTS

The research results were obtained by comparing the diameter of the antibacterial inhibition zones of citronella essential oil from Bengkulu against the bacteria *Enterococcus faecalis* ATCC 29212 and S*taphylococcus aureus* ATCC 25923 using the disk diffusion method or Kirby-Bauer method.

All collected and recorded data were subsequently analyzed statistically using IBM SPSS 29.0.2.0 data processing and statistical software. Before conducting the comparison tests, the first statistical analysis performed was a normality test using the Shapiro-Wilk test, as the sample size was less than 50. The results of the Shapiro-Wilk normality test showed $p > 0.05$ in all eight groups, indicating that all data are normally distributed. The next step was to perform a parametric test using One-way ANOVA. The results of the One-way ANOVA test and the average antibacterial inhibition tests can be seen in Tables 1 and 2.

Table 1. One-Way ANOVA test on *Enterococcus faecalis*

Test Group	Inhibition Zone *Enterococcus faecalis* (mm)					Mean (mm) ± SD	Sig
	1	2	3	4	5		
Citronella Essential oil 50%	2,45	1,6	1,6	0,4	1,34	1,34 ± 0,82	<0,001
Citronella Essential oil 75%	1,2	1,4	1,55	1,5	2,4	1,61 ± 0,46	
Citronella Essential oil 100%	2,3	2,65	2,9	2	2,95	2,56 ± 0,40	
CHX 2%	6,25	4,45	4,2	3,35	5,05	4,66 ± 1,07	

Table 2. One-Way ANOVA test on *Staphylococcus aureus*

Test Group	Inhibition Zone					Mean (mm) ± SD	Sig
	1	2	3	4	5		
Citronella Essential oil 50%	0,60	1,20	1,45	1,80	3,30	1,67 ± 1,01	<0,001
Citronella Essential oil 75%	0,50	1,00	1,10	1,15	1,35	1,02 ± 0,31	
Citronella Essential oil 100%	1,15	1,20	1,50	1,80	1,35	1,59 ± 0,47	
CHX 2%	5,45	5,75	5,75	6,00	6,80	5,95 ± 0,51	

Based on the results of the tests conducted on the eight treatment groups, the results showed significance <0.001 ($p<0.05$), indicating that there is a significant difference in antibacterial inhibition between citronella essential oil from Bengkulu at concentrations of 50%, 75%, and 100%, as well as CHX 2% as the positive control.

The statistical analysis was then continued with the Post Hoc Bonferroni test to examine and determine the significantly different treatment groups. The results from the Post Hoc Bonferroni test analysis show significant differences between all citronella oil groups and the positive control CHX 2% ($p<0.001$). The comparison between the 50% concentration and the 75% concentration yielded a p-value of 1.000, while the comparison between the 75% and 100% concentrations produced a p-value of 0.366, and the comparison between the 100% and 50% concentrations resulted in a p-value of 0.119. This suggests that the differences among the treatment groups are not significant against *Enterococcus faecalis*. Based on the findings regarding the antibacterial effectiveness of citronella essential oil (*Cymbopogon nardus*) from Bengkulu against *Enterococcus faecalis* ATCC 29212, it can be concluded that the concentrations that most effectively inhibit bacterial growth are 100%, 75%, and 50%.

In contrast, the citronella essential oil from Bengkulu at a concentration of 50% compared to the 75% concentration does not show a significant difference, with a significance value of 0.750. The citronella essential oil from Bengkulu at a concentration of 75% compared to the 100% concentration also does not show a significant difference, with a significance value of 1.000. Similarly, the comparison between the 100% concentration and the 50% concentration also does not indicate a significant difference, with a significance value of 1.000. However, in terms of magnitude, the order of concentrations of citronella essential oil with the largest inhibition zone against *Staphylococcus aureus* ATCC 25923 is 50%, followed by 100%, and then 75%.

4. DISCUSSION

This study was conducted with the aim of determining the inhibitory effect of Bengkulu citronella essential oil on the growth of bacteria after root canal treatment. The citronella essential oil used in this study was a finished product whose contents had been tested. The main components found in the essential oil were citronellol, citronellal at 50.09%, and geraniol at 81.89%. Other antibacterial components included saponins, flavonoids, polyphenols, alkaloids, terpenoids, tannins, and essential oils. This study aligns with research conducted by Dwi et al. in 2021, which found that citronella contains saponins, flavonoids, polyphenols, alkaloids, and essential oils, all of which have antibacterial properties.[12]

Enterococcus faecalis and *Staphylococcus aureus* were selected as samples in this study because these gram-positive cocci pathogens are frequently found as causes of root canal treatment (RCT) failure. These bacteria are among the resistant species that cause periradicular disease and pulp necrosis, as they can survive the effects of root canal treatment and persist as pathogens within the root canal and dentinal tubules.[4,6,23] *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 used in this study were bacterial cultures obtained from the MiCore Laboratory of the Faculty of Dentistry, Trisakti University.

The results of the antibacterial inhibition test of citronella essential oil against *Enterococcus faecalis* (Figure 1) and *Staphylococcus aureus* (Figure 2) in this study showed that there was an inhibitory effect on bacterial growth at concentrations of 50%, 75%, and 100%. This can be observed from the clear zones formed around the paper discs on the agar medium.

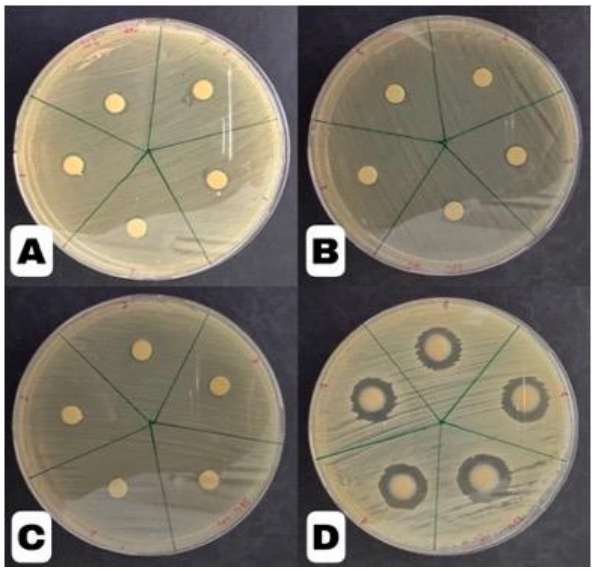

Figure 1. Research Results of the Antibacterial Inhibition Test of Citronella Essential Oil against *Enterococcus faecalis*. 100% Concentration (A), 75% Concentration (B), 50% Concentration (C), 2% CHX (D).

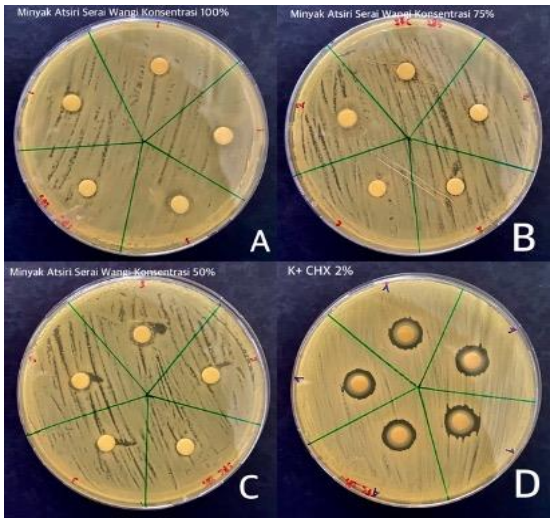

Figure 2. Research Results of the Antibacterial Inhibition Test of Citronella Essential Oil against *Staphylococcus aureus*. 100% Concentration (A), 75% Concentration (B), 50% Concentration (C), 2% CHX (D).

According to Davis & Stout (1971), antibacterial activity is categorized based on the clear zone that forms. The categories are divided based on the measurement of the inhibition zone diameter formed around the paper disc. If the diameter of the inhibition zone formed is less than 5 mm, it is considered weak; if the diameter is between 5 – 10 mm, it is considered moderate; if the diameter is between 10 – 20 mm, it is considered strong; and if the diameter is greater than 20 mm, it is categorized as very strong. Based on the results of the above study, the antibacterial activity of citronella essential oil at concentrations of 50%, 75%, and 100% falls into the weak category.[31]

Factors that influence the antibacterial effectiveness of essential oils include concentration, the type of solvent used, distillation method, quality of raw materials, and storage methods.[7,20] The essential oil used in this study was distilled in May 2024. It was stored by tightly sealing the essential oil container and placing it in a cool location away from direct sunlight. The choice of solvent for the essential oil was also determined to ensure the oil could dissolve and be prepared at specific concentrations.

Citronella essential oil has an antibacterial mechanism by disrupting the formation and exerting a toxic effect on the structure and function of bacterial cell walls or membranes. This is due to the ability of its compounds to move from the polar phase to the non-polar bacterial cell membrane.[15] The size of the inhibition zone produced may be influenced by the diffusion capacity of the essential oil. The thicker the essential oil concentration tested, the more difficult it becomes to diffuse, and this can also be affected by the solubility level between the essential oil and the diluent when dissolved using a vortex.

According to Afrizal et al. 2024 study, the compounds in citronella essential oil can cause changes in cell membrane permeability by removing membrane proteins.[33] This study is consistent with research by Udawaty et al. in 2019, which found that citronella essential oil has antibacterial effects against *Enterococcus faecalis* growth.[34] Meanwhile, research conducted by Sefriyanti et al. showed that citronella essential oil can inhibit the growth of *Staphylococcus aureus*.[13] The study by Howarto et al. in 2015 demonstrated that a 100% concentration was the most effective in inhibiting *Enterococcus faecalis*.[8] This study is also in line with Afrizal et al.'s 2024 findings, which showed that citronella essential oil has an antibacterial effect on *Staphylococcus aureus* at a 50% concentration.[33]

According to research by Gumelar et al. in 2022, the duration of withering of citronella can affect the yield of the content produced after the distillation process. The chopping process before distillation is also one of the factors influencing the essential oil content.[35] This is supported by research conducted by Juliarti et al. in 2020, which found that the planting pattern used can also affect the yield of essential oil.[36] A study by Dacosta et al. in 2017 mentioned that essential oil content from plants grown at higher altitudes is greater than those grown in lowland areas. Differences in altitude can also affect the secondary metabolite content in essential oils.[37]

This is reinforced by research by Sari et al. in 2022, which stated that antibacterial activity can be influenced by several factors, including technical factors, biological factors, the content of antibacterial compounds, the diffusion strength of the test material, and the type of bacteria used. Technical factors include the amount of inoculum, incubation time and temperature, the composition and thickness of the agar medium. Biological factors, which are beyond the researcher's control, relate to bacterial resistance properties. The content of antibacterial compounds is related to the

environment where the plants grow, with differences in conditions such as temperature, light intensity, humidity, rainfall, and soil type and nutrients available, all affecting the production of secondary metabolites. Therefore, the plant's growth location significantly influences the type and quantity of secondary metabolites found in the plant.[15]

Based on theory, previous research, and the results of this study, it can be concluded that the antibacterial inhibition activity of Bengkulu citronella essential oil (*Cymbopogon nardus*) is influenced by the content of secondary metabolites in the essential oil, its concentration, and its diffusion capacity. Additionally, the antibacterial activity is affected by the type of bacteria being inhibited, the concentration of the inoculum, incubation temperature, incubation time, media composition, and media thickness.

5. CONCLUSIONS

Citronella (*Cymbopogon nardus*) essential oil from Bengkulu has antibacterial activity against the growth of *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923. A 100% concentration is most effective in inhibiting *E. faecalis* ATCC 29212, while a 50% concentration is most effective in inhibiting *S. aureus* ATCC 25923. The inhibitory effect observed can be categorized as low, as the inhibition zone is less than 5 mm.

REFERENCES

I. Anonymous. *Riset Kesehatan Dasar Tahun 2018*. Jakarta: Kementerian Kesehatan Republik Indonesia, 2020:182.

II. Anonymous. *Situasi Kesehatan Gigi dan Mulut 2019*. Jakarta: Kementerian Kesehatan Republik Indonesia; 2020.

III. Garg N, Garg A. *Textbook of Endodontics*. 4th Ed. India: Jaypee Brothers Medical Publishers; 2019:22,111-113,236-237,239,241.

IV. Gopikrishna V. *Grossman's Endodontic Practice*. 14th Ed. India: Wolters Kluwer Health; 2021:14,38-40,226,265-266,310,315-316.

V. Berman LH, Hargreaves KM. *Cohen's Pathways of The Pulp*. 12th Edition. Missouri; Elsevier. 2021:28–30,284.

VI. Torabinejad M, Fouad AF, Shabahang S. *Endodontic Principles and Practice*. 6th Edition. Missouri; Elsevier. 2021:69–72,321.

VII. Pasril Y, Yuliasanti A. Antibacterial Power of Red Betel Leaves (Piper Crocatum) To Enterococcus Faecalis Bacteria As Medicament Material For Root Canal By Dilution Method. *Insisiva Dental Journal*. 2014;1(3):88–94.

VIII. Howarto MS, Wowor PM, Mintjelungan CN. Uji Efektivitas Antibakteri Minyak Atsiri Sereh Dapur Sebagai Bahan Medikamen Saluran Akar Terhadap Bakteri *Enterococcus Faecalis*. *Jurnal E-Gigi (Eg)*. 2015;3(2):432–438.

IX. Rotstein I, Ingle JI. *Ingle's Endodontics 7*. North Carolina: PMPH USA; 2019:657–659.

X. Hargreaves KM, Berman LH. *Cohen's Pathway of the Pulp*. 11th Ed. Missouri: Elsevier; 2016:260–262.

XI. Kambaya PP, Jumiati, Masyhudi. Uji Efek Antibakteri Ekstrak Etanol Buah Belimbing Wuluh (Averrhoa Bilimbi L) Sebagai Kandidat Bahan Medikamen Saluran Akar Gigi Terhadap Pertumbuhan *Staphylococcus Aureus* Secara In Vitro. *Mulawarman Dental Journal*. 2021;1(1):2.

XII. Dwi RS, Tanjung D, Chairani S, Biker Indrasari R, Dewi Sr. Evaluation Of Antibacterial Activity Of Cymbopogon Nardus L. On The Growth Of Enterococcus Faecalis. *Jurnal Kedokteran Gigi*. 2021;15(1):24–29.

XIII. Sefriyanti, Jayuska A, Alimuddin AH, editors. Uji Aktivitas Antibakteri Minyak Atsiri Serai Wangi (*Cymbopogon bernadus L*.) Terhadap Bakteri Escherichia Coli Dan *Staphylococcus Aureus*. *Jurnal Kimia Khatulistiwa*. 2020;4(8):1.

XIV. Nurcholis W, Takene M, Puspita R, Tumanggor L, Qomaliyah EN, Sholeh MM, editors. Antibacterial Activity of Lemongrass (Cymbopogon nardus) Ethanolic Extract against *Escherichia coli* dan *Staphylococcus aureus. Curr. Biochem*. 2019;2(6):88–91.

XV. Sari I, Misrahanum, Faradilla M, Ayuningsih CM, Maysarah H. Antibacterial Activity of Citronella Essential Oil from *Cymbopogon nardus* (L.) Rendle) Against Methicillin-Resistant *Staphylococcus aureus*. *Indonesian Journal of Pharmaceutical and Clinical Research (IDJPCR)*. 2022;5(1):16–22.

XVI. Tanjung DS, Wijaya S, Silaen M, editors. Efektivitas antibakteri ekstrak daun serai (Cymbopogon citratus) konsentrasi 20%, 30%, 40%, dan 50% terhadap *Streptococcus mutans*. *Prima Journal of Oral and DentalSciences*. 2022;1(5):21.

XVII. Fitriani L, Tuntun M, Marhamah, editors. Uji Daya Hambat Ekstrak Serai Wangi (*Cymbopogon nardus* (L.) Rendle) Terhadap Pertumbuhan Bakteri Escherichia Coli ATCC 29522. *Jurnal Medika Malahayati*. 2021;3(5):192,197.

XVIII. Patel S, Barner JJ. *The Principles of Endodontics.* 3rd Edition. UK: Oxford University Press. 2020:80.

XIX. Schoch CL, Ciufo S, Domrachev M, et al. NCBI Taxonomy: a comprehensive update on curation resources and tools. *Database*. 2020.

XX. Soedarto. *Mikrobiologi Kedokteran*. Jakarta: Sagung Seto. 2015:194.

XXI. Kumar S. *Essentials of Microbiology*. 1st Edition. New Delhi: Jaypee Brothers Medical Publishers. 2016:163-164.

XXII. Ernawati L, Sumantri S, Wedagama W, Astuti A, Fatmasari D. Effectiveness Of Black Cumin Extract (*NigellaSativa*) In Inhibiting The Growth Of *Staphylococcus Aureus* Bacteria In Vitro. *Jurnal Kesehatan Gigi.* 2023;10(1):56–63.

XXIII. Yamin IF, Natsir N. Dominant Bacteria In Root Canal Of Necrotic Teeth. *Dentofasial Journal*.2014;13(2):113-116

XXIV. Yuanita T, Ceson MAB, Subiyanto A. Differences in Effectiveness of Antibacterial Power Between Cocoa Peel Extract (*Theobroma cocoa* L.) and Benzalkonium Chloride 0.1% Against *Staphylococcus aureus* (In Vitro). *Conservative Dentistry Journal*. 2021;11(2):56-61.

XXV. Pasril Y, Okasari D. Pengaruh Daya Anti Bakteri Ekstrak Bunga Mawar Merah (*Rosa damascene Mill*) Terhadap Pertumbuhan *Enterococcus faecalis. Insisiva Dental Journal.* 2020;9(1):28.

XXVI. Nurbianti AA, Alhawaris, Yani S. Daya Hambat Ekstrak Etanol Batang Akar Kuning (*Arcangelisia flava (L) Merr*) Terhadap Pertumbuhan Bakteri *Streptococcus mutans, Porphyromonas gingivalis,* dan *Enterococcus faecalis*. *Mulawarman Dental Journal*. 2021;2(1):79

XXVII. Tenda PE, Lenggu MY, Ngale MS. Antibacterial Activity Test of Ethanol Extract of Faloak Tree Skin (*Sterculia sp.*) On *Staphylococcus Aureus* Bacteria. *Jurnal Info Kesehatan*. 2017;1(15):234.

XXVIII. Sulaswatty A, Rusli MS, Abimanyu H, Tursiloadi S. *Qua Vadis Minyak Serai Wangi dan Produk Turunannya.* Jakarta: LIPI PRESS. 2019:19,43-50.

XXIX. Soraya C, Sunnati, Wulandari F. Efek Antibakteri Ekstrak Daun Mimba (*Azadirachta indica*) Terhadap Pertumbuhan Bakteri *Enterococcus faecalis* Secara In-Vitro. *Cakradonya Dent J*. 2019;1(11):24–25.

XXX. Nurhayati LS, Yahdiyani N, Hidayatulloh, A. Perbandingan Pengujian Aktivitas Antibakteri Starter Yogurt dengan Metode Difusi Sumuran dan Metode Difusi Cakram. *Jurnal teknologi Hasil Peternakan.* 2020;1(2):41-46.

XXXI. Wijaya S, Susanto C, Zuardi F. Uji Aktivitas Antibakteri Ekstrak Kulit Lemon (*Citrus limon*) konsentrasi 6,25%, 12,5%, 25%, dan 50% terhadap Bakteri *Enterococcus faecalis*. *Prima Journal of Oral and Dental Sciences.*2022;1(5):46.

XXXII. Sari DP, Ichrom MY, Budiarti LY. Efektivitas Daya Hambat Ekstrak Umbi Bawang Dayak Terstandarisasi Fenol terhadap Pertumbuhan *Enterococcus faecalis*. *Dentino*. 2017;1(1):59.

XXXIII. Afrizal, Defitriani A, Efdi M. Minyak Atsiri Serai Wangi (*Cymbopogon nardus* L. *Rendle*): Diisolasi Dengan Dua Metode Berbeda, Kualitas dan Aktivitas Antibakterinya. *Journal Riset Kimia*. 2024;15(1):99-111.

XXXIV. Udawaty W, Yusro F, Sisillia S. Identifikasi Senyawa Kimia Minyak Sereh Wangi Klon G3 (*Cymbopogon nardus L.*) dengan Media Tanam Tanah Gambut dan Potensinya sebagai Antibakteri *Enterococcus faecalis*. *Jurnal Tengkawang*. 2019;2(9):78-79.

XXXV. Gumelar AM, Ersan, Supriyatdi D. Pengaruh Lama Pelayuan dan Pencacahan Daun Serai Wangi (*Cymbopogon winterianus Jowitt ex Bo*r) pada Rendeman dan Mutu Citronella Oil. *Jurnal Agro Industri Perkebunan*. 2022;1(10):7.

XXXVI. Juliarti A, Wijayanto N, Mansur I, Trikoesoemaningtyas. Analisis Rendeman Minyak Sereh Wangi (*Cymbopogon nardus* L.) yang Ditanam dengan Pola Agroforestri dan Monokultur pada Lahan Revegetasi Pasca Tambang Batubara. *Jurnal Sylva Lestari*. 2020;2(8):187.

XXXVII. Dacosta M, Sudirga SK, Muksin IK. Perbandingan Kandungan Minyak Atsiri Tanaman Sereh Wangi (*Cymbopogon nardus L. Rendl*) yang Ditanam Di Lokasi Berbeda. *Jurnal Simbiosis V*. 2017;1(5):30.