ORIGINAL ARTICLE

Effectivity of *Kaempferia Galanga L.* Essential Oil Against *Streptococcus Pyogenes* and *Streptococcus Sanguinis* for Root Canal Medicament

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ABSTRACT

Introduction: Root canal treatment for pulp necrotic aimed to eliminate pulp inflammation and restoring tooth function. Inflammation in this area mostly by gram-positive facultative anaerobic bacteria which are *Streptococcus pyogenes* and *Streptococcus sanguinis*. To eliminate all bacteria in the root canal it can be allocated medicaments to keep it sterile until procedure filling the root canal. It is highly possible that essential oil from *Kaempferia galanga Linn* potential to be used as an antibacterial root canal medicaments. **Methods:** Experimental laboratory research with post-test only in the control group. The group divided into two arms of *S. pyogenes* and *S. Sanguinis* and subsequently divided into 8 groups with concentration of 6.25%; 7.25%; 8.25%; 9.25%; 10.25%; 11.25%; 12.25%; 12.5% and 6 groups of concentration (4%, 5%, 6%, 7%, 8%, 9%) respectively. **Results:** The MIC of *S. pyogenes* were 8,25% compared to 6% of *S.sanguinis*, while the MBC were 9.25% and 7%, respectively. Data analysis showed a significant difference in the concentration of essential oil from *Kaempferia galanga L.* on the number of colonies *S.pyogenes* and *S. sanguinis* (One way ANOVA, sig = 0.000) and there was a significant correlation with each increase in the concentration of essential oil of *Kaempferia galanga L.* which decreased the number of colonies *S.pyogenes* and *S. sanguinis* (Pearson, sig= 0.000, Correlation coefficient= -0.841; -0.899). **Conclusion:** Essential oil of *Kaempferia galanga Linn.* potentially effective for root canal medicament against both in vitro study.

Keywords: Root canal treatment; *Kaempferia galanga L.* rhizome essential oil; *Streptococcus pyogenes*; *Streptococcus sanguinis*

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INTRODUCTION

Dental caries is a progressive form of tooth mineral loss, progressive over time, with less remineralization process than demineralization (19). Demineralization is closely related to the level of acidity and duration of the acidity level on the tooth surface (28). Ultimate aspects which trigger dental caries are the host (teeth and saliva), substrate (food), caries-inducing microorganisms and time (29). Treatment suggestively performs immediately on the affected area, delayed action will lead to pulp necrosis due to penetration of bacterial toxins into the pulp and root canals (4). Pulp necrosis is an irreversible condition characterized by the destruction of both hard and soft tissues of the tooth (5).

Up to 90% of bacteria that dominate root canals are anaerobic due to conditions which are suitable for bacterial growth and gram-positive organisms (22,23). Common bacteria found in this site are Streptococcus spp. 25%, including 16.5% *Streptococcus pyogenes* and up to 88% *Streptococcus sanguinis* (1,13,21,34,). One treatment option to treat pulp necrosis is Root Canal Treatment (RCT) (30). This method is targeted to treat inflammation and to maintain teeth function. Protocols for RCT include preparation for cleaning and shaping, sterilization in terms of disinfection and irrigation, and obturation of the root canal. Bacteria presence contributed to the effectiveness of the root canal treatment. The presence of resistant bacteria after RCT and bacteria that multiply after root canal obturation can lead to root canal treatment failure (31). Therefore, medicament materials are needed to increase the chance of success in these modalities(27).

Medicaments refer to substances used to reduce

pain in the root canal, eliminate bacteria in the root canal and prevent secondary infection (31). It is known various medicament materials such as calcium hydroxide, phenol groups, aldehydes, halides, steroids, antibiotics, chlorhexidine digluconate, and poly-antimicrobial paste (7,17-18). However, the chemical compounds contained in medicament ingredients are usually accompanied by the side effects of active allergen and are toxic for users (6,31).

Traditional ingredients for medicines are considered safer because relative minimal side effects (7). One of this traditional ingredients is *Kaempferia galanga L.* which has active ingredients of flavonoids, saponins, polyphenols, alkaloids, terpenoids, and essential oils that potentially have antibacterial roles (14,24,29). One of the main ingredients of kencur rhizome is essential oil (10). The essential oil of kencur rhizome has the main and the most content up to 54.07%, namely ethyl p-methoxycinnamate (12). Ethyl p-methoxycinnamate is a chemical compound that has antibacterial activity (9).

Testing of *Kaempferia galanga L.* essential oil as a root canal medicament against *Streptococcus pyogenes* and Streptococcus sanguinis has never been done, so researchers need to prove that has Kaempferia galanga L. essential oil effectiveness as a root canal medicament against *Streptococcus pyogenes* and Streptococcus sanguinis in vitro study.

MATERIALS AND METHODS

Research Design

This study applied a laboratory experimental research design that included only post-test in the control group.

Research Sample

The sample in this study used a random sampling technique of bacteria:
(1) *Streptococcus pyogenes*
(2) *Streptococcus sanguinis*

Research Variables

The independent variable on the bacterium *S. pyogenes* treated with essential oil of aromatic ginger rhizome in different concentration n of 6.25%; 7.25%; 8.25%; 9.25%; 10.25%; 11.25%; 12.25%; 12.5%, and *S. sanguinis* bacteria treated with essential oil of aromatic ginger rhizome in a concentration of 4%; 5%; 6%; 7%; 8%; 9%. The dependent variable refers to the number of bacterial colonies of *S. pyogenes* and *S. sanguinis.*

Research Procedure

a. Test Suspension Preparation
Two bacterial colonies were strake on BHIB (Brain Heart Infusion Broth) media and growth in an incubator for 24 hours at 37°C. Subsequently the BHIB media containing growth bacteria were measured for its Optical Density (OD) using a spectrophotometer at max = 625 nm wavelength. The colony measures were immersed into a suspension containing 10^8 CFU/ml and diluted by mixing 9 ml of 0.9% NaCl using the formula N1 x V1 = N2 x V2 to obtain 10^6 CFU/ml. The 1 ml from the two bacteria suspensions were taken and dropped into the prepared test tube.

b. Negative and Positive Controls
Negative control prepared by mixing 10% DMSO (Dimethyl Sulfoxide) solution and 0.5% Tween 80 in ratio of 1:1 based on calculations (example 1 ml of negative control solution is needed):

Volume DMSO 10% = 0,5 ml
Volume Tween 80 0,5% = 0,5 ml

Positive control was made by mixing 1 ml of Cresotin liquid No.2 and 1 ml of *S. pyogenes* bacterial suspension and mixing 1 ml of 2% Chlorhexidine and 1 ml of *S. sanguinis* bacterial suspension.

c. Antibacterial Activity of Aromatic ginger Rhizome Essential Oil against *Streptococcus pyogenes*
Sterile tubes were provided for *S. pyogenes* and *S. sanguinis* colonies on BHIB media and equalized with a spectrophotometer for its turbidity. A preliminary study performed to find the concentration density, the assay biologically triplicates. An aromatic ginger (known as aromatic ginger) rhizome essential oil with various concentrations was prepared as before.

Bacterial suspension with a concentration of 10^6 CFU/ml was prepared. One ml bacterial suspension poured into all tubes and incubated for 24 hours at 37°C. After 24 hours, MIC (Minimum Inhibitory Concentration) value was measured by visually observing the turbidity of the tube. One loop taken for inoculation on BHIA media before incubating for 24 hours at 37°C.

On the third day, the MBC (Minimum Bactericidal Concentration) value was obtained from colony counting. MBC is determined by inhibition of bacterial colony proliferation on the BHIA media.

Data Analysis

Firstly, the normality and homogeneity tests were used to determine the effect of aromatic ginger rhizome different concentrations on the growth of *S. pyogenes* and S.sanguinis colonies. One-Way ANOVA test was used to determine the effect of different concentrations of the aromatic ginger rhizome essential oil on the growth of *S. pyogenes* and S.sanguinis colonies.

Additionally, Pearson correlation analysis was used to determine the relationship between increasing aromatic ginger rhizome essential oil concentration and decreasing S.pyogenes and S.sanguinis colony counts. The effect of giving the aromatic ginger rhizome essential oil on the growth of *S. pyogenes* and *S. sanguinis* colonies was then determined using a multivariate linear regression analysis.

RESULTS

Prior antibacterial activity test with the core concentration, a preliminary test was conducted to determine the maximum concentration.

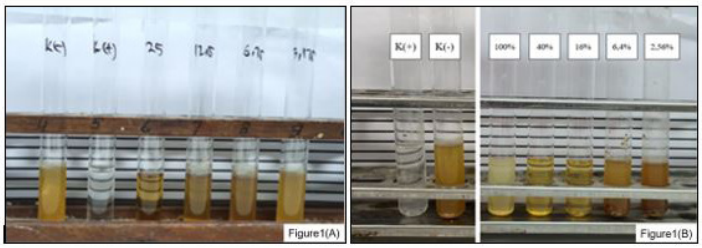

Figure 1 : Different turbidity on the preliminary test tube dilution using aromatic ginger rhizome essential oil on (A) *S pyogenes.* (B) *S. sanguinis*. (Personal documentation, 2020).

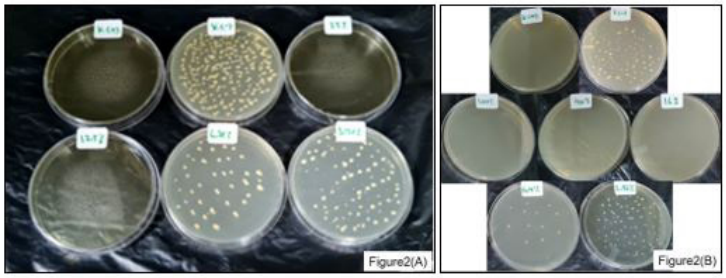

Figure 2 : Preliminary test streaking results on BHIA media (A) *S. pyogenes*. (B) *S.sanguinis.* (Personal documentation, 2020).

After 24 hours of incubation on *S. pyogenes* bacteria, the preliminary test tube dilution demonstrated that concentration of 12.5 percent apparently clear, while for *S. sanguinis* demonstrated clear turbidity concentration of 16 percent. BHIA culture media for *S. pyogenes* bacteria obtained a concentration of 12.5 percent, which resulted in no bacterial growth, and a concentration of 6.25 percent, which resulted in continued bacterial growth. In *S. sanguinis*, the 16 percent concentration was no longer detected, but the 6.4 percent concentration was still breeding bacteria. Thus, the concentration was compressed to determine the MIC and MBC.

At 37°C, the tube dilution test with core concentration was incubated for 24 hours. The MBC value is determined by scratching the tube dilution results onto a plate containing BHIA media and streaking results indicate that there is no bacterial proliferation in *S. pyogenes* at a concentration of 9.25 percent and no bacterial proliferation in *S. sanguinis* at a concentration of 7 percent.

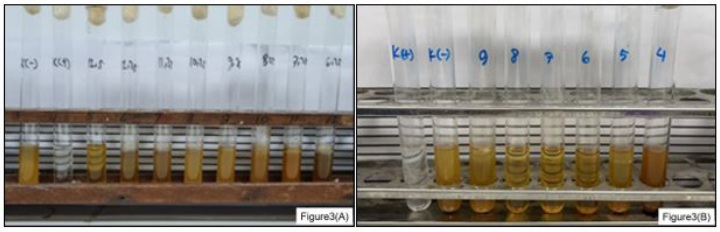

Figure 3 : Dilution results of the test tube concentration of the essential oil of aromatic ginger rhizome essential oil (A) *S. pyogenes* with MIC 8,25% (B) Streptococcus sanguinis with MIC 6%. (Personal documentation, 2020).

The antibacterial test results were repeated three times for each treatment. Furthermore, bacterial colonies in each BHIA medium were counted using a colony counter.

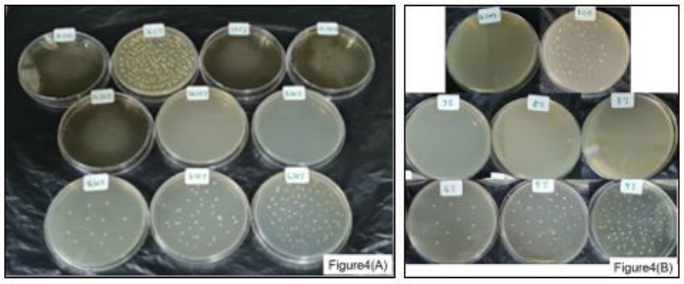

Figure 4 : Streaking results of aromatic ginger rhizome essential oil on BHIA media (A) *S. pyogenes* with MBC 9,25%. (B) *S. sanguinis* with MBC 7%. (Personal documentation, 2020).

Based on the results of different tests, the effect of giving various concentrations of the aromatic ginger rhizome essential oil on the decrease in the growth of the number of *S. pyogenes* and *S. sanguinis* colonies resulted in a significant 0.000. The value of sig < 0.05 means that there is a significant difference in the average concentration of each concentration of the aromatic ginger rhizome essential oil on the number of colonies of *S. pyogenes* and *S. sanguinis*.

From the results of the Pearson correlation test, the value of sig = 0.000 was obtained which stated that there was a significant correlation between the increase in the concentration of the aromatic ginger rhizome essential oil and the proliferation of *S. pyogenes* and *S. sanguinis* colonies.

Table I : (A). Simple Linear Regression Test Results Data *S. pyogenes*

Variable	Unstandardized Coefficients		Standardized Coefficiens	t	Sig.
	B	Std.Error	Beta		
Constanta	87,892	10,571		8,314	0,000
Concentration of Kaempferia Essential Oil	-7,786	1,068	-0,841	-7,287	0,000

Table I : (B). Simple Linear Regression Test Results Data *S. sanguinis*

Variable	Unstandardized Coefficients		Standardized Coefficiens	t	Sig.
	B	Std.Error	Beta		
Constanta	86,187	8,841			
Concentration of Kaempferia Essential Oil	-10,781	1,316	-0,899	-8,195	0,000

The r count obtained from the *S. pyogenes* test was -0.841 and from the *S. sanguinis* test was -0.899. The negative result indicates that the higher the concentration of the aromatic ginger rhizome essential oil, the lower the number of *S. pyogenes* and *S. sanguinis* colonies. In the range of relationship strength, the calculated r value obtained from the *S. pyogenes* bacteria test was in the strong range, and from the *S. sanguinis* bacterial test was in the very strong range.

Table I. Simple Linear Regression Test Results Data (A) *S. pyogenes* (B) *S. sanguinis*

From the results of the regression test on the *S. pyogenes* bacteria test, the following equation was obtained:

Y = 87,892 – 7,786 the concentration of essential oil of the aromatic ginger rhizome

The results of the regression test on the *S. sanguinis* bacteria test obtained the following equation:

Y = 86,187 – 10,781 the concentration of essential oil of the aromatic ginger rhizome

This equation can be interpreted as follows:
a. The constant of the *S. pyogenes* test equation was 87.892 and the *S. sanguinis* test was 86.187. This explains the average number of colonies of *S. pyogenes* and *S. sanguinis* if no essential oil is given.
b. The regression coefficient states that the average number of colonies of *S. pyogenes* bacteria will decrease by 7.786 and that of *S. sanguinis* by 10.781 at each application of 1 percent concentration of the aromatic ginger rhizome essential oil.

The magnitude of the effect of the concentration of aromatic ginger rhizome essential oil on bacterial proliferation was obtained, the variable concentration of aromatic ginger rhizome essential oil in predicting the growth of bacterial colonies of *S. pyogenes* was 0.7 percent and *S. sanguinis* was 80.8 percent, while other factors were affected by other variables that were not included in this study.

DISCUSSION

The isolates of *S. sanguinis* and *S. pyogenes* which were implemented in this study were obtained from the Microbiology Research Center Laboratory of the Faculty of Dentistry, Airlangga University which has an accredited statement letter through gram staining and biochemical tests. Gram stain test with bacterial staining technique to distinguish gram positive and gram negative bacteria (25). Gram-positive bacteria showed purple results (8). In both these bacteria, purple results were obtained which indicated that these bacteria were Gram Positive bacteria (35). The microbiological analysis method used is TPC (Total Plate Count), the basic principle behind this approach is to embed live microbial cells in agar media, so that they will grow and combine into colonies that can be seen and counted visually (33).

Making essential oil of kencur rhizome (*Kaempferia galanga L.*) using steam distillation method. Distillation

is a method of purification of solid compounds starting from the evaporation of liquid compounds through a heating process, then the vapor formed will be accommodated in a separate container to obtain ure liquid compounds(20). The solvent for kencur rhizome essential oil used in this study was DMSO 10% and Tween 80 0.5%(15).

Dimethyl Sulfoxide (DMSO) has the capacity to penetrate cell membranes. The use of DMSO concentration as a solvent is prohibited from exceeding 10% because it can trigger cell membranes to lysis (2). Tween 80 is a trademark of an ester compound that acts as an emulsifier that can increase the solubility of essential oils. Based on preliminary tests that have been carried out by previous researchers, that 10% DMSO + Tween 80 0.5% solution does not have antibacterial activity, so the solution can be used as a negative control(15).

The Minimum Inhibitory Concentration (MIC) of kencur rhizome essential oil against *S. pyogenes* was 8.25% and *S. sanguinis* was 6%. Previous research on *S. pyogenes* also revealed a reduction in bacterial reproduction by red ginger essential oil which is still of the same order Zingiberales with *Kaempferia galanga L.*, that is, an antibacterial effectiveness of 5% was obtained due to the presence of chemical compounds in red ginger, namely 1-3% essential oil, oleoresin, terpenoids, gingerols and shogaols (3), while previous studies on *S. sanguinis* showed reduced bacterial proliferation by cinnamon essential oil (Cinnamon cassia), which was obtained antibacterial effectiveness at a concentration of 5% due to the presence of cinnamaldehyde and eugenol compounds (11).

The significance value of the ANOVA test results of 0.000 ($p < 0.05$) showed that there were differences in treatment at each concentration of kencur rhizome essential oil to decrease the number of colonies of *S. pyogenes* and *S. sanguinis*. The increase in the concentration of kencur rhizome essential oil is directly proportional to the increase in chemical compounds which include saponins, flavonoids, phenols, alkaloids, tannins, ethyl p-methoxycinnamate, and monoterpenoids. Thus, causing differences in the effectiveness of kencur rhizome essential oil (12,14,19,24,26).

The higher the concentration of kencur rhizome essential oil that was applied, the breeding of *S. pyogenes* and *S. sanguinis* colonies would decrease. The decrease in the proliferation of bacterial colonies of *S. pyogenes* and *S. sanguinis* was due to the presence of saponin activity that damaged the permeability of the bacterial membrane (14). Flavonoids can inhibit bacterial DNA synthesis resulting in disrupted bacterial metabolism (26). Tannins combine with proteins to form H+ ions and cause the pH to turn acidic, rendering the bacterial enzymes inactive. Alkaloids work by preventing the formation of bacterial cell walls (16). Ethyl p-methoxycinnamate is a phenol derivative compound. Phenol and protein will create hydrogen bonds that can destroy the protein structure of bacteria. The hydrogen bonds will affect the permeability of the cell wall and the bacterial cytoplasmic membrane which consists of proteins. Disruption of the permeability of the bacterial cell wall and cytoplasmic membrane will trigger an imbalance of macromolecules and ions in bacterial cells, resulting in cell lysis. Monoterpenoids as antibacterial are the result of reactions with proteins outside the bacterial cell wall membrane, thereby creating polymeric bonds that can damage proteins. Damage to proteins will result in disruption of the penetration of compounds into the bacterial cell wall, can trigger bacterial cells to lose nutrients and slow bacterial reproduction and even die (19).

The effect of giving kencur rhizome essential oil to decrease the number of colonies of *S. pyogenes* by 70.7% and *S. sanguinis* by 80.8%. While other factors that can affect the growth of the number of bacterial colonies include physical and chemical aspects. Physical aspects include pH and osmotic pressure. Meanwhile, the chemical aspects include light, sulfur, phosphorus, carbon and nutrients in the growth medium (32).

CONCLUSION

Kaempferia galanga L. essential oil potentially effective for root canal medicament against both bacteria species in this in vitro study.

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REFERENCES

1. Al-Hamadani AH, Al-Yasiri RK, Manky MA, Al-Jannat MA. Evaluation of The Antimicrobial Effect of Endodontic Sealers on Microbiota Associated with Root Canal Infections. QMJ. 2011;7(12):1-12.
2. Andayani R, Mubarak Z, Rinanda DR. Aktivitas Antibakteri Tepung Cacing Tanah (Lumbricus rubellus) Terhadap Enterococcus faecalis Secara In

Vitro. JDS. 2016;1(2):201–210.
3. Awanis MA, Mutmainnah AA. Uji Anti Bakteri Ekstrak Oleoresin Jahe Merah (Zingiber officinale var. rubrub) Terhadap Bakteri *Streptococcus pyogenes*. Jurnal Ilmiah Kedokteran. 2016;3(1):33-41.
4. Banerjee A, Watson TF. Pickard's Manual of Operative Dentistry (9th ed.). Oxford University Press. 2011.
5. Beer R, Baumann MA, Kielbassa AM. Atlas Saku Endodontik. Jakarta: EGC; 2015.
6. Chong BS. Harty's Endodontics in Clinical Practice (7th ed.). Elsevier. 2016.
7. Cohenca N. Disinfection of Root Canal Systems: The Treatment of Apical Periodontitis (1st ed.). John Wiley & Sons, Inc. 2014.
8. Dewi ZY, Nur A, Hertriani T. Efek Antibakteri dan Penghambatan Biofilm Ekstrak Sereh (Cymbopogon nardus L.) Terhadap Bakteri Streptococcus mutans. Majalah Kedokteran Gigi Indonesia. 2015;1(2):136–141.
9. Fajeriyati N, Andika. Uji Aktivitas Antibakteri Ekstrak Etanol Rimpang Kencur (*Kaempferia galanga L.*) Pada Bakteri Bacillus subtilis dan Escherichia coli. Journal of Current Pharmaceutical Sciences. 2017;1(1):36–41.
10. Hajanajumudin H, Satari MH, Setiawan AS. Antibacterial Effect of Cinnamon Essential Oil (Cinnamon cassia) in Different Concentration Towards Streptococcus sanguis. Padjadjaran Journal of Dentistry. 2010;22(2):256-262.
11. Hasanah AN, Nazaruddin F, Febrina E, Zuhrotun A. Analisis Kandungan Minyak Atsiri dan Uji Aktivitas Antiinflamasi Ekstrak Rimpang Kencur (*Kaempferia galanga L.*). Jurnal Matematika & Sains. 2011;16(3):147-152.
12. Inagaki Y, Abe M, Inaki R, Zong L, Suenaga H, Abe T, Hoshi K. A Case of Systemic Infection Caused by Streptococcus pyogenes Oral Infection in an Edentulous Patient. MDPI. 2017;5(3):1-6. DOI: 10.3390/diseases5030017.
13. Kharismayanti A, Wahyukundari MA, Ermawati T. Uji Aktivitas Antibakteri Minyak Atsiri Daun Jeruk Nipis (Citrus aurantifolia (Christm. & Panz.) Swingle) Terhadap Porphyromonas gingivalis ATCC 33277 Secara In Vitro (5th ed.). PROSDING (DSMoJ V). 2018;91-100.
14. Lely N, Rahmanisah D. Uji Daya Hambat Minyak Atsiri Rimpang Kencur (Kaempferia galanga Linn) Terhadap Trichophyton mentagrophytes, Trichophyton rubrum. JPS MIPA UNSRI. 2017;19(2):94-99.
15. Marselia S, Wibowo MA, Arreneuz S. Aktivitas Antibakteri Ekstrak Daun Soma (Ploiarium alternifolium melch) Terhadap Propionibacterium acnes. Jurnal Kimia Khatulistiwa. 2015;4(4):72–82.
16. Mattulada IK. Pemilihan Medikamen Intrakanal Antar Kunjungan yang Rasional. Dentofasial. 2010;9(1):63-68.
17. McCabe JF, Walls AWG. Bahan Kedokteran Gigi (9th ed.). EGC. 2014.
18. Meuthia N. Uji Hambat Antibakteri Minyak Atsiri Rimpang Kencur (*Kaempferia galanga L.*) Terhadap Pertumbuhan Methicillin-Resistant Staphylococcus aureus. Skripsi. 2015:1-69.
19. Mount GJ, Hume WR, Ngo HC, Wolff MS. Preservation and Restoration of Tooth Structure (3rd ed.). John Wiley & Sons, Ltd. 2016.
20. Mustiadi L, Astuti S, Purkuncoro AE. Buku Ajar Distilasi Uap dan Bahan Bakar Pelet Arang Sampah Organik. CV. IRDH. 2020.
21. Nugroho SW, Rukmo M, Prasetyo EA, Tamara Y. Antibakteri Ekstrak Kulit Buah Kakao (Theobroma cacao) 6,25% dan NaOCl 2,5% Terhadap Bakteri Streptococcus sanguinis. Conservative Dentistry Journal. 2019;9(1):19-21.
22. Posangi J, Juliatri, Bara R, Tairas J, Wuisan J. Uji Efek Antibakteri Tinta Cumi-cumi (Loligo sp.) Terhadap Bakteri Saluran Akar Gigi. 2013:1-6.
23. Priyanka SR, Veronica. Flare-Ups in Endodontics-A Review. IOSR-JDMS. 2013;9(4):26-31.
24. Ratu AP, Mugiyanto E. Uji Toksisitas Daun Ketepeng (Cassia Alata L.), Kulit Buah Pisang Ambon (Musa Paradisiaca L. Var Sapientum) dan Kulit Rimpang Kencur (Kaempferia Galanga Linn.) Dengan Metode Brine Shrimp Lethality Test (BSLT). URECOL. 2018:189-194.
25. Saputera MMA, Marpaung TWA, Ayuchecaria N. Konsentrasi Hambat Minimum (KHM) Kadar Ekstrak Etanol Batang Bajakah Tampala (Spatholobus littoralis Hassk) Terhadap Bakteri Escherichia coli Melalui Metode Sumuran. Jurnal Ilmiah Manuntung. 2019;5(2):167-173.
26. Sari DP, Ichrom NMY, Budiarti LY. Efektivitas Daya Hambat Ekstrak Umbi Bawang Dayak Terstandarisasi Fenol terhadap Pertumbuhan Enterococcus faecalis. DENTINO JKG. 2017;1(1):56-61.
27. Shahani MN, Subba RVV. Comparison of Antimicrobial Substantivity of Root Canal Irrigants in Instrumented Root Canals an in Vitro Study. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2011;29(1):28–33. DOI: 10.4103/0970-4388.79925.
28. Sibarani MR. Karies: Etiologi, Karakteristik Klinis dan Tatalaksana. Majalah Kedokteran Universitas Kristen Indonesia. 2014;30(1):14–22.
29. Sina MY. Khasiat Super Minuman Alami Tradisional Beras Kencur dan Kunyit Asem. Diandra Pustaka Indonesia. 2016.
30. Soleh, Megantara S. Karakteristik Morfologi Tanaman Kencur (*Kaempferia galanga L.*,) dan

Aktivitas Farmakologi. Farmaka. 2019;17(2):256-262.
31. Torabinejad M, Walton RE, Fouad AF. Endodontics Principles and Practice (5th ed.). Saunders. 2014.
32. Tortora GJ, Funke BR, Case CL. Microbiology: An Introduction (10th ed.). CA : Pearson Benjamin Cummings. 2010.
33. Wati RY. Pengaruh Pemanasan Media Plate Count Agar (PCA) Berulang Terhadap Uji Total Plate Count (TPC) di Laboratorium Mikrobiologi Teknologi Hasil Pertanian UNAND. Jurnal TAMAPELA. 2018;1(2):44–47.
34. Yamin IF, Natsir N. Bakteri Dominan di Dalam Saluran Akar Gigi Nekrosis. Dentofasial. 2014;13(2):113-116.
35. Yonathan, Dwiandhany SW. Perawatan Endodontik Regeneratif Pada Gigi Matur Nekrosis Dengan Atau Tanpa Kelainan Periapikal: Kajian Pustaka. Makassar Dent J. 2019;8(1):46-50.