ANTIBACTERIAL EFFECT OF KELAKAI LEAF EXTRACT (*STENOCHLAENA PALUSTRIS (BURM) BEDD.*) FOR INHIBITING *ENTEROCOCCUS FAECALIS*

*I Wayan Arya Krishnawan Firdaus *, Nurdiana Dewi**, Ridwan Ichshalul Fuady***, Maharani Laillyza Apriasari*****

**Department of Oral Biology, Faculty of Dentistry, Lambung Mangkurat University*
***Department of Pediatric Dentistry, Faculty of Dentistry, Lambung Mangkurat University*
****Faculty of Dentistry, Lambung Mangkurat University*
*****Department of Oral Medicine, Faculty of Dentistry, Lambung Mangkurat University*

***Correspondence*: iwayan.firdaus@ulm.ac.id**

Keywords:

Enterococcus faecalis; Inhibition activity; Kelakai leaf extract (Stenochlaena Palustris (Burm) Bedd.)

ABSTRACT

Background: Root canal treatment is a stage of treating pulp infection by removing the necrotic tissue and eliminating microorganisms. Inadequate sterilization cause persistent root canal bacteria, including Enterococcus faecalis. The irrigation solution that has become the gold standard in root canal treatment is sodium hypochlorite but it has some weaknesses. Kelakai leaf extract can be an alternative root canal irrigation because it has minimal side effects and antibacterial compounds such as flavonoid, saponin, alkaloid, and tannin.
Methods: This research was a true experimental laboratory with posttest only and control group design. The research using 5 treatment groups with 3 replications, so that total sample was 15 samples. Group 1-4 were kelakai leaf extract concentrations of 25%, 50%, 75%, 100% and group 5 was Sodium hypochlorite 2,5%. The parameter measured was the diameter of the inhibition zone (mm) formed on MHA.
Results: One Way Anova and Post-Hoc LSD test results showed a significant difference between each treatment group of kelakai leaf extract compared with Sodium hypochlorite 2,5%. Kelakai leaf extract 25%, 50%, 75%, 100% concentrations, and Sodium hypochlorite 2,5% had an average inhibition zone diameter which were 9.47 mm, 14.64 mm, 17.91 mm, 21.24 mm, and 23.27 mm.
Conclusion: Kelakai leaf extract (Stenochlaena palustris (Burm) Bedd.) concentrations of 25%, 50%, 75%, and 100% had inhibitory activity against Enterococcus faecalis.

INTRODUCTION

Dental and oral health problems in the society are generally caused by infection of the pulp tissue. In 2014, pulp and periapical disease was ranked 4th in the 10 most common diseases in Banjarmasin with a total of 29,898 cases.[1] Root canal treatment is the choice in the treatment of pulp infections with the aim of removing necrotic tissue, eliminating microorganisms, and accelerating healing of periapical lesions.[2] Root canal treatment is divided into three stages, namely preparation, sterilization, and obturation. Sterilization is one of the important factors that affect the success of root canal treatment. Inadequate sterilization results in the retention of persistent bacteria in the root canals, including *Enterococcus faecalis* bacteria.[3]

Enterococcus faecalis is a facultative anaerobic gram-positive bacterium, which can reproduce and colonize in areas with or without oxygen. These bacteria are opportunistic, usually

associated with failure root canal treatment and the most common causes of nosocomial infections.[4] Nearly 90% of cases of secondary root canal infection are caused by *Enterococcus faecalis*.[5] *Enterococcus faecalis* has the ability to penetrate the dentinal tubules, form a biofilm in the root canal, and survive in conditions of low nutrient intake. These bacteria can be eliminated by performing root canal irrigation.[3]

Sodium hypochlorite (NaOCl) has been widely accepted as the gold-standard irrigation solution since its introduction in endodontics in 1936. NaOCl reduces surface tension thereby facilitating the release of debris from the root canal wall.[6] NaOCl bind with H_2O to form hypochlorous acid (HClO) compounds as a strong oxidizing agent associated with antibacterial activity. In addition, NaOCl can also dissolve tissue and the presence of the sodium element of this material acts as a lubrication.[7] However, this material also has several weaknesses including high toxicity which causes cell damage, unpleasant odor, and corrosion of endodontic appliances, so alternative efforts are needed as an irrigation solution using herbal ingredients that have antibacterial activity.[8] One of the herbal plants that can be used because it has antibacterial activity is the kelakai plant (*Stenochlaena palustris* (Burm) Bedd.).[9]

Indonesia has abundant sources of biological wealth, especially on the island of Borneo because of its tropical climate. The plant that thrives in the Kalimantan area is the kelakai plant (*Stenochlaena palustris* (Burm) Bedd.) which is commonly found in peat swamp areas. The society uses this plant as a vegetable and as traditional medicine because it has antibacterial properties and an affordable price.[10] The kelakai plant (*Stenochlaena palustris* (Burm) Bedd.) contains flavonoid, saponin, alkaloids, tannin, and steroid.[11] The content of the Kelakai leaves water extract has a high total flavonoid content, which is 503.56 mg QE/g.[12]

Several studies have proven the kelakai leaf extract as an antibacterial. Pertiwi (2019) tested the antibacterial activity of the ethanol kelakai leaf extract at concentrations of 25%, 50%, 75%, and 100% against *Streptococcus sanguinis* bacteria. The inhibition zone produced by the ethanol kelakai leaf extract started from a concentration of 25% and the largest inhibition zone was found at a concentration of 100% with a diameter of 16.32 mm.[9] Rostinawati (2017) conducted a research of antibacterial activity test of ethanol kelakai leaf extract against *Staphylococcus aureus* which mentioned that the MIC is at a concentration of 10.6% and MBC is at a concentration of 11%.[10]

Based on the description above, it can be seen that the ethanol kelakai leaf extract is able to inhibit the growth of *Streptococcus sanguinis* and *Staphylococcus aureus* which are facultative anaerobic gram-positive bacteria. The kelakai leaf extract was used to reduce the effects of *Sodium hypochlorite*, such as high toxicity, unpleasant odor, and corrosion of endodontic appliances. There is no research on the kelakai leaf extract as an irrigation solution, so it is necessary to conduct research on the antibacterial effect of kelakai leaf extract (*Stenochlaena palustris* (Burm) Bedd.) for inhibiting Enterococcus faecalis.

MATERIAL AND METHOD

This type of research was a true experimental laboratory using a Posttest-only with control group design. The research has obtained an ethical license and research permit by the Research Ethics Commission of the Faculty of Dentistry, Lambung Mangkurat University No. 017/KEPG-FKGULM/EC/II/2021. This research used 5 treatments derived from the concentration of 25%, 50%, 75%, 100% kelakai leaf extracts

(*Stenochlaena palustris* (Burm) Bedd.), and *Sodium hypochlorite* 2.5% as positive control. The total of replications based on the *Lemeshow* formula calculation was 3 times. The research took place at the Basic Laboratory of the Faculty of Mathematics and Natural Science, the Biochemistry Laboratory of the Faculty of Medicine, and the Biomedical Laboratory of the Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin, starting from January–March 2021.

The materials used in this research included kelakai leaf extract, ethanol 90%, aquades, kalium dichromate, *Enterococcus faecalis* ATCC 19433, Trypton Yeast Cystein (TYC) media, Brain Heart Infusion Broth (BHI-B) media, Mueller Hinton Agar (MHA) media, alcohol 70%, 0.5 McFarland standard solution, sterile paper disk, and *Sodium hypochlorite* 2.5%. The tools used in this research include analytical scale, blender (panasonic), extractor, WH40 filter paper, Erlenmeyer flask, shaker, oven, rotary evaporator (Heidolp), water bath (SMIC), anaerobic incubator, autoclave, petri dish, pipette drops, ose, measuring cup, rack and test tube, micropipette, mask, handscoon, laminary air flow, marker, bunsen lamp, lighter, millimeter scale calliper, and sterile cotton swab.

The production of kelakai leaf extract began with picking the kelakai leaf in Anjir area, Barito Kuala Regency, South Kalimantan. 2 kg of kelakai leaf were taken with criteria for mature leaf, namely green, rough leaf surface and ± 11 cm long. The kelakai leaf were washed and dried at room temperature. The leaf were put in the oven for 4 hours at 40ºC, then blended and sifted to become simplicia powder. Extraction was carried out using the maceration method, which was mixing simplicia powder with 96% ethanol solution while stirring using a shaker for 1 x 24 hours in order to make the active compound of the kelakai leaf soluble with 96% ethanol. The extract solution was filtered and evaporated with a rotary evaporator at a temperature of 50-60°C for 4-6 hours, then the extract was heated over a water bath to obtain 18 g of the pure extract of the kelakai leaf. The extract was then tested for ethanol free by adding kalium dichromate ($K_2Cr_2O_7$). Then, the kelakai leaf extract was diluted with aquades until concentrations of 25%, 50% and 75% were obtained. The concentration dilution can be calculated using the following formula:

$$\mathbf{V_1 \times C_1 = V_2 \times C_2}$$

Information:

V_1 = baseline volume (ml)

C_1 = baseline concentration (%)

V_2 = final volume (ml)

C_2 = final concentration (%)

This research used *Enterococcus faecalis* ATCC 19433 bacterial isolate obtained from MBRIO Food Laboratory, Bogor. Bacterial isolates of *Enterococcus faecalis* were grown on Trypton Yeast Cystein (TYC) media and then incubated for 1x24 hours at 37°C. Bacteria were inoculated into 5 ml of liquid BHI and incubated for 2x24 hours at 37°C in an anaerobic incubator. The bacterial suspension on liquid BHI media was diluted until the turbidity was comparable to the standard Mc Farland 0.5 (1.5x108 CFU/ml) and smeared on Mueller Hinton Agar media with a sterile cotton swab.

The research on the inhibitory activity of the kelakai leaf extract with *Sodium hypochlorite* 2.5% against *Enterococcus faecalis* was conducted using the diffusion method. Paper disks were immersed in kelakai leaf extract solution of 25%, 50%, 75%, 100% concentrations and sodium hypochlorite

2.5% for 24 hours. Then, paper disks were placed on MHA media containing bacteria and incubated at 37°C for 24 hours. The zone of inhibition of bacterial growth was measured using a calliper in mm.

RESULTS

The results of the inhibition zones from the concentration of 25, 50%, 75% and 100% of the extract with *Sodium hypochlorite* 2.5% against *Enterococcus faecalis* on the test media can be seen in figure 1.

Table 1. showed that there were differences in inhibition zone variations from the treatment of extracts of 25%, 50%, 75%, 100% and 2.5% s*odium hypochlorite* on the growth of *Enterococcus faecalis* bacteria with 3 replications. The results of the research can be seen to have a trend of increasing the average size of the resulting inhibition zone. The increase in the concentration of the kelakai leaf extract can increase the size of the inhibition zone. The results showed that the 100 % concentration of kelakai leaf extract had a larger average inhibition zone value of 21.24 mm, compared to the inhibition zone values of 25%, 50%, and 75% concentrations. However, the 100 % concentration of kelakai leaf extract had a smaller inhibition zone value compared to s*odium hypochlorite* 2.5% which had an inhibition zone value of 23.27 mm.

The data that had been collected from each treatment was then tabulated and normality test was performed using the *Saphiro-Wilk*, the data was normally distributed if p>0.05. The results of the normality test of *Saphiro-wilk* kelakai leaf extract and s*odium hypochlorite* 2.5% on the growth of *Enterococcus faecalis* bacteria obtained p>0.05, which means that the data is normally distributed. The data was then tested for homogeneity using *Levene's test* and obtained a significance value of 0.086 (p>0.05), which means that the variance between groups is homogeneous.

The research data were normally distributed and homogeneous and fulfilled the requirements for further analysis, namely the *One Way Anova* parametric test with a 95% confidence level. The results of the *One Way Anova* parametric test obtained a value of p = 0.000 (p <0.05) which indicated a significant difference in each treatment group of kelakai leaf extract and s*odium hypochlorite* 2.5% on the growth of *Enterococcus faecalis* bacteria. Data analysis was continued with the *Post-Hoc Least Significant Difference* (LSD) test to find out which groups had significant differences. The results of the LSD test can be seen in Table 2.

Based on the results of the Post-Hoc LSD test, each treatment group has a significance value of 0.000 (p<0.05), which means that from each treatment group, the concentration of kelakai leaf extract 25%, 50%, 75%, 100% and *Sodium hypochlorite* 2,5% had a significant difference in the size of the inhibition zone of *Enterococcus faecalis* bacteria.

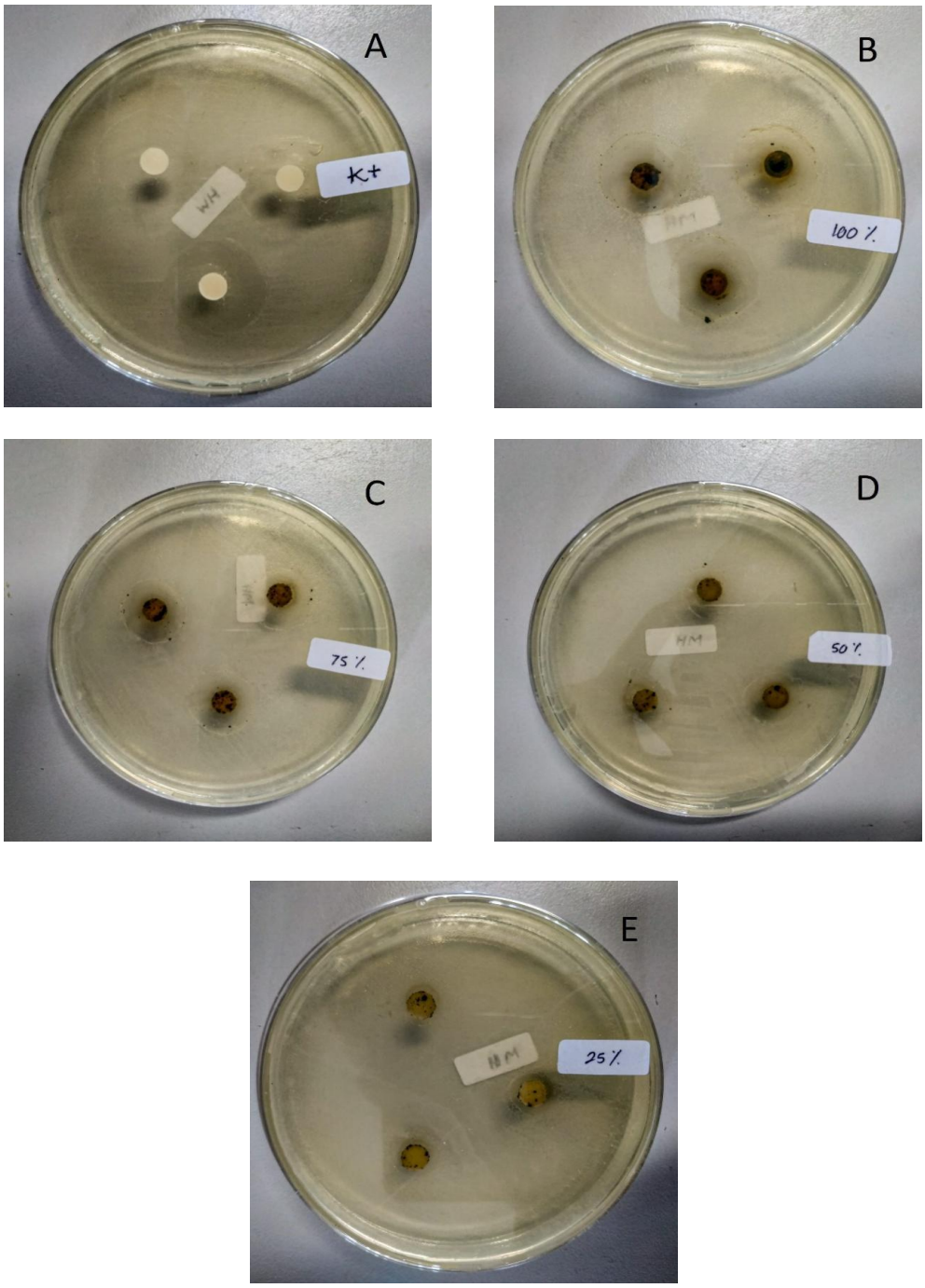

Figure 1. Inhibitory Zone Formed From Each Treatment; (A) Sodium hypochlorite 2.5%, Kelakai Leaf Extract Concentration (B) 100%, (C) 75%, (D) 50%, and (E) 25% In Each Replications.

Table 1. The Average of Inhibition Zone of Kelakai Leaf Extract with Sodium hypochlorite 2.5% against Enterococcus faecalis.

	Average Inhibition Zone (mm)				
	EDK 25%	**EDK 50%**	**EDK 75%**	**EDK 100%**	**NaOCl 2,5%**
1	9,86	14,90	17,93	20,90	23,43
2	8,80	14,80	18,06	21,73	23,36
3	9,76	14,23	17,76	21,10	23,03
***Mean* ± SD**	9,47 ± 0,58	14,64 ± 0,36	17,91 ± 0,15	21,24 ± 0,43	23,27 ± 0,21

Information:
EDK : Kelakai Leaf Extract
NaOCl 2,5% : *Sodium hipoklorit* 2,5%

Table 2. Post Hoc Test Results of LSD Kelakai Leaf Extract and 2.5% Sodium hypochlorite against Enterococcus faecalis

Perlakuan	EDK 25%	EDK 50%	EDK 75%	EDK 100%	NaOCl 2,5%
EDK 25%		0,000*	0,000*	0,000*	0,000*
EDK 50%	0,000*		0,000*	0,000*	0,000*
EDK 75%	0,000*	0,000*		0,000*	0,000*
EDK 100%	0,000*	0,000*	0,000*		0,000*
NaOCl 2,5%	0,000*	0,000*	0,000*	0,000*	

* : Significant differences (p<0,05)

DISCUSSION

Based on the research, the kelakai leaf extract (*Stenochlaena palustris*) was proven to inhibit *Enterococcus faecalis* bacterial growth. These results are in line with Soraya's (2019) research on neem leaf extract with concentrations of 10%, 20%, 40%, 60%, 80%, and 100% which were proven to inhibit the growth of *Enterococcus faecalis*.[13]

Enterococcus faecalis is a facultative anaerobic gram-positive bacterium, which is characterized as a bacterium that is able to survive in unfavorable conditions for these bacteria in the root canal, including extreme environmental conditions and long-term nutritional deficiencies.[3] Virulence factors that play a role in the pathogenesis of *Enterococcus faecalis* consist of several components, namely Aggregation Substanse (AS), Cytolysin, Surface adhesins, Lipoteichoic Acid (LTA), Sex pheromones, Extracellular Superoxide Production (ESP), Hyaluronidase, and Gelatinase lytic enzyme. These virulence factors cause *Enterococcus faecalis* to have various abilities, including the ability to compete with other bacteria, form colonization, damage tissues, induce inflammation, and resistance to root canal medicaments.[14]

The results of the LSD test showed that there was a significant difference in the inhibition zone between the treatment group of kelakai leaf extract (concentration 25%, 50%, 75% and 100%) and *Sodium hypochlorite* 2.5% in inhibiting the growth of *Enterococcus faecalis* bacteria. The results of this research showed an increase in the mean zone of inhibition along with the increase in the concentration of the kelakai leaf extract on the growth of *Enterococcus faecalis* bacteria. Based on Nisa's research (2017), it is stated that the increase in the average inhibition zone of *Enterococcus faecalis* is directly proportional to the increase in the concentration of the given extract. This is due to an increase in the active substance or secondary metabolites contained in it along with the increase in the concentration of the extract.[15]

This research used ethanol 96% as solvent which has a high level of polarity so that it can dissolve secondary metabolite compounds in the kelakai leaf (*Stenochlaena palustris*) which have the same polarity, namely flavonoid, saponin, alkaloid, tannin, and steroid.[11] Flavonoid is the most common secondary metabolites contained in the kelakai leaf extract.[12] Bacterial growth can be inhibited by flavonoids because it can form complexes with proteins through hydrogen bonding. [16] The function of flavonoid in inhibiting the growth of *Enterococcus faecalis* has a mechanism by inhibiting the function of the bacterial cytoplasmic membrane by forming complex compounds with extracellular proteins so it can damage the cytoplasmic membrane of bacterial cells followed by the release of intracellular compounds, loss of cations and macromolecules from cells, cause disruption of cell growth and bacterial cell death, this causes the growth of *Enterococcus faecalis bacteria* were inhibited.[17,18]

The next metabolite compound of kelakai leaf that act against the *Enterococcus faecalis* bacteria is saponin. The mechanism of action of saponin in inhibiting the growth of *Enterococcus faecalis* bacteria is by lowering the surface tension so there is an increase in cell permeability and leakage which causes intracellular substances such as organic ions, amino acids, and nutrients out of the bacterial cells. According to Haniastuti (2016), saponin is also a strong surfactant agents that will be absorbed by the bacterial cell surface, an increase in membrane permeability will eliminate essential ingredients such as enzyme and protein in bacteria.[19]

Alkaloid play a role in inhibiting the growth of bacteria by interfering with the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not fully formed and is at risk of bacterial cell lysis. Herawati et al (2017) stated that *Enterococcus faecalis* is a gram-positive bacterium that structurally has a thick peptidoglycan layer, so that it has a higher level of sensitivity to compounds that have the potential to inhibit or damage bacterial cell wall synthesis.[20]

The ability of tannin to act against bacteria is by inactivating the microbial cell adhesin.[20] Mubarak et al (2016) stated that tannin have antibacterial activity because their toxicity can damage bacterial cell membrane. This is because the toxicity process will form metal ion from tannin which have various functions, such as can shrink the cell wall and then interfere with the permeability of the bacterial cell itself so that the metabolism of the bacterial cell is disrupted, growth is inhibited, and lysis.[21]

Sodium hypochlorite 2.5% had a higher mean inhibition zone than kelakai leaf extract concentration of 100%. Based on Mozartha's

research (2019) which compared the antibacterial activity of Curcuma zedoaria extract and s*odium hypochlorite* 2.5% against *Enterococcus faecalis*, it was stated that the control group using s*odium hypochlorite* 2.5% had the largest mean diameter of the inhibition zone compared to the extract group.[8]

The reaction between tissue organic matter and s*odium hypochlorite* will result in a neutralization reaction of amino acids and a chloramination reaction by NaOH and HClO. *Sodium hypochlorite* neutralizes amino acids by releasing hydroxyl ions, then converting them into water and salt through a neutralization reaction, resulting in a decrease in pH. Hypochlorous acid (HClO) solution which is a strong oxidizing agent through chlorine compounds will oxidize hydrosulfuric compounds found in the essential bacterial enzyme, namely cysteine. This process results in the inhibition of the enzyme work of the bacterium *Enterococcus faecalis*, then cell metabolism and the integrity of the cytoplasmic membrane will be disrupted. The chlorine compound then reacts with the amine group on the surface of the bacterial protein which will produce chloroamine compound (NH_2Cl) as a biofilm dispersant that can degrade *Enterococcus faecalis* biofilm.[7] Hypochlorous acid causes a potential reaction through an oxidation-reduction reaction that produces several new compounds such as superoxide, hydrogen peroxide, hydroxyl radicals and oxygen, most of which are mutagenic to bacteria, resulting in protein denaturation, lipid oxidation in membrane or cell wall, deactivation of enzyme and causing damage to bacterial DNA.[22]

CONCLUSION

Kelakai leaf extract (*Stenochlaena palustris* (Burm) Bedd.) concentrations of 25%, 50%, 75%, and 100% has inhibitory activity against *Enterococcus faecalis*.

REFERENCES

1. Badan Pusat Statistik (BPS). *Jumlah Kasus 10 Penyakit Terbanyak di Kota Banjarmasin.* 2020 Dec 1. Available from https: //banjarmasinkota.bps.go.id/statictable/2015/12/09/652/jumlah-kasus-10-penyakit terbanyak -di-kota-banjarmasin-2014.html.
2. Sari DP, Nahzi MYI, Budiarti LY. Efektivitas Daya Hambat Ekstrak Umbi Bawang Dayak Terstandarisasi Fenol terhadap Pertumbuhan *Enterococcus faecalis*. *Dentino Jurnal Kedokteran Gigi*. 2017; 1(1): 56-61.
3. Mahendra I, Wardani I, Rochyani L. Daya Antibakteri Ekstrak Ikan Teri Jengki (*Stolephorus Insularis*) terhadap *Enterococcus faecalis*. *Denta Jurnal Kedokteran Gigi.* 2018; 12(2): 106-116.
4. Jangnga ID, Kambaya PP, Kosala K. Uji Aktivitas Antibakteri Dan Analisis Bioautografi Kromatografi Lapis Tipis Ekstrak Etanol Daun Srikaya (Annona Squamosa L) Terhadap Enterococcus Faecalis Secara In Vitro. ODONTO Dental Journal. 2018; 5(2): 102-109.
5. Subrata A, Prahasti AE, Iskandar BO. Influence of Two Root Canal Obturation Techniques with Resin Based Sealer to *Enterococcus faecalis* Penetration. *Journal of Indonesian Dental Association*. 2019; 2(1): 21-28.
6. Kovac J, Kovac D. Effect of Irrigating Solutions in Endodontic Therapy. Bratisi Lek Listy. 2011. 112(7): 410-5.
7. Widiastuti D, Karima IF, Setiyani E. Efek Antibakteri *Sodium hypochlorite* terhadap *Staphylococcus aureus. Jurnal Ilmiah Kesehatan Masyarakat.* 2019; 11(4): 302-307.
8. Mozartha M, Silvia P, Sujatmiko B. Perbandingan Aktivitas Antibakteri Ekstrak Curcuma *zedoaria* dan Bahan Irigasi Natrium Hipoklorit 2.5% terhadap *Enterococcus faecalis*. *Jurnal Material Kedokteran Gigi.* 2019; 8(1): 22-29.
9. Pertiwi FC, Firdaus IWAK, Erlita I. Comparison of Inhibitory Activity of Kelakai Leaf Extract and 0.2% *Chlorhexidine Gluconate* against *S.Sanguinis*. *Dentino Jurnal Kedokteran Gigi*. 2019; 4(2): 145-150.
10. Arullappan S, Sawai S, Chee L, Mahandan M, Shanmugavelan R. Phytochemical Screening and Evaluation of Cytotoxic Effect and Antioxydant Activity of Fractions Isolated from Stenochlaena palustris (Burm) Bedd. (Burm.f) Bedd Leaves. Indian Journal of Pharmaceutical Education and Research. 2017; 51(45): 735-737
11. Nurhasnawati H, Sundu R, Sapri, dkk. Antioxidant Activity, Total Phenolic and Flavonoid Content of Several Indigenous Species of Ferns in East Kalimantan,

Indonesia. *Biodiversitas*. 2019; 20 (2): 578-580.
12. Chear N, Kooi-Yeong K, Vikneswaran M, Choon-Sheen L. Cholinesterase Inibitory Activity and Chemical Constituents of *Stenochlaena palustris* Fronds at Two Different Stages of Maturity. *Journal of Food and Drug Analysis*. 2016; 24: 366-358.
13. Soraya C, Sunnanti, Wulandari F. Efek Antibakteri Ekstrak Daun Mimba (*Azadirachta indica*) terhadap Pertumbuhan *Enterococcus faecalis* Secara *In-Vitro*. *Cakradonya Dental Journal*. 2019; 11(1): 23-32.
14. Noviyandri PR, Andayani R, Rizky E. Potensi Ekstrak Alga Merah *Gracilaria verrucosa* Sebagai Penghambat Perkembangan Pembentukan Biofilm *Enterococcus faecalis* Pada Infeksi Saluran Akar Gigi. *Jurnal Syiah Kuala Dentistry Society*. 2018; 3(1): 6-13.
15. Nisa R, Erlita I, Budiarti LY. Aktivitas Daya Hambat Ekstrak Etanol Daun Belimbing Wuluh Dan Sodium Hipoklorit terhadap *Enterococcus faecalis* (In Vitro). *Dentino Jurnal Kedokteran Gigi*. 2017; 2(2): 200-204.
16. Rahman EF, Christiono S. Effectivity Antibacterial Zinc Oxide Eugenol With Zinc Oxide Propolis For Endodontic Treatment In Primary Teeth. ODONTO Dental Journal. 2019; 6(2): 113-117.
17. Harseno S, Mooduto L, Prasetyo EP. Daya Antibakteri Ekstrak Daun Kedondong Bangkok (*Spondias dulcis* Forst.) terhadap Bakteri *Enterococcus faecalis*. *Conservative Dentistry Journal.* 2016; 6(2): 110-116.
18. Setiawan AS, Prisinda D, Fatriadi F. Aktivitas Antibakteri Fraksi Etanol Daun Kemangi (Ocimum Americanum) Terhadap Enterococcus Faecalis Atcc 29212. ODONTO Dental Journal. 2020; 7(2): 111-116.
19. Haniastuti T. Penurunan Hidrofobisitas Permukaan Sel Bakteri Plak Gigi Setelah Dipapar Rebusan Daun Sirih Merah Konsentrasi 10%. *Dentika Dental Journal*. 2016; 19(1): 38-41.
20. Herawati L, Noviayandri PR, Nasution AI. Pengaruh Ekstrak Buah Timun Suri (*Cucumis sativus L*.) sebagai Antibakteri Alami dalam Menghambat Pertumbuhan *Enterococcus faecalis*. *Journal Caninus Dentistry*. 2017; 2(3): 111-116.
21. Mubarak Z, Chismirina S, Daulay HH. Aktivitas Antibakteri Ekstrak Propolis Alami Dari Sarang Lebah terhadap Pertumbuhan *Enterococcus faecalis*. *Journal of Syiah Kuala Dentistry Society*. 2016; 1(2): 175-186.
22. Rahardia N, Rulianto M, Wahjuningrum DA. Perbedaan daya antiglukan NaOCl 2,5% dan ekstrak kulit manggis (*Garcinia mangostana L*.) 0,09% terhadap *Enterococcus faecalis. Conservative Dentistry Journal.* 2017; 7(1): 1-5.