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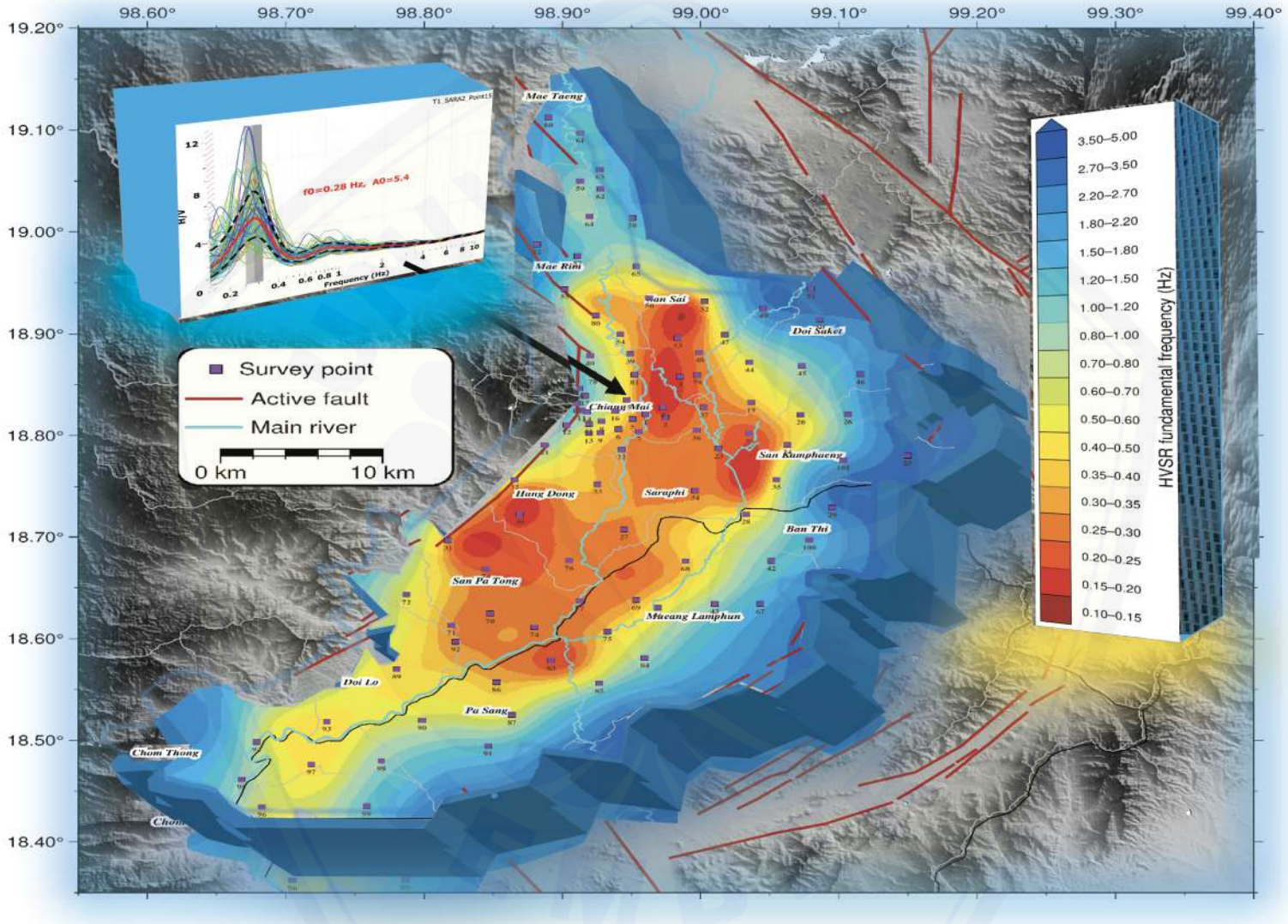

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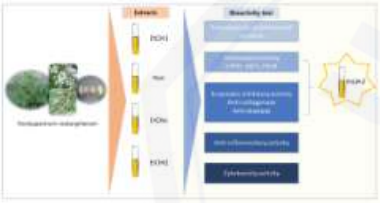

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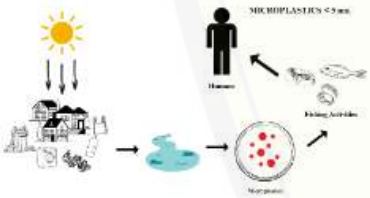

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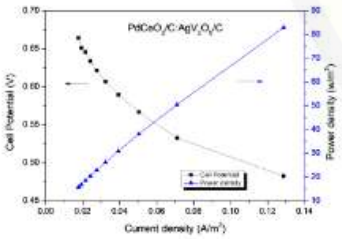

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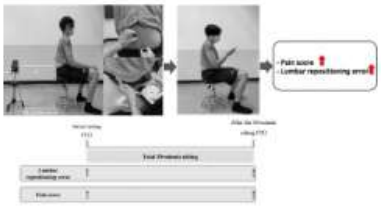

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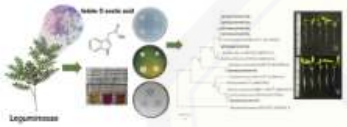

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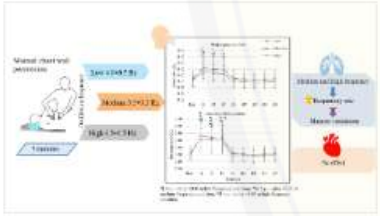

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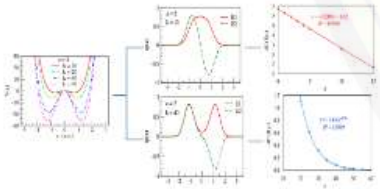

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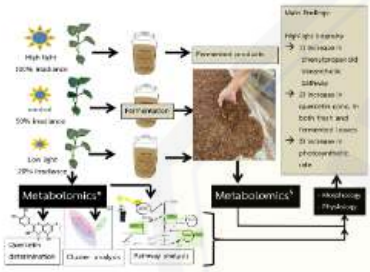

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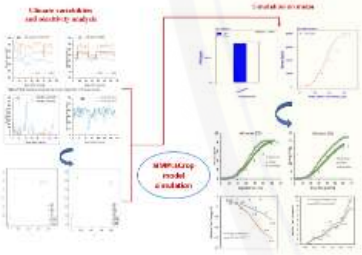

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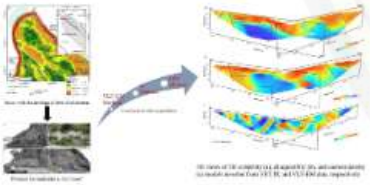

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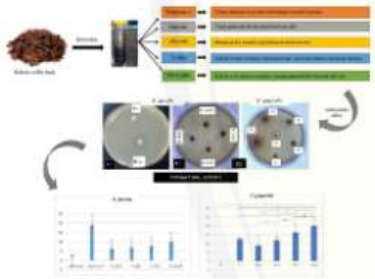

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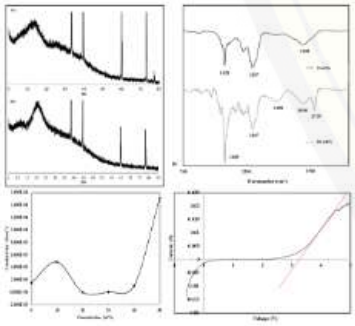

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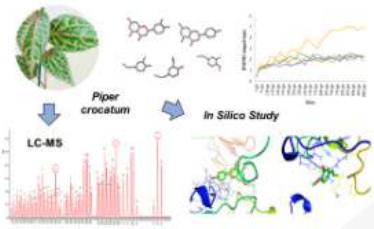

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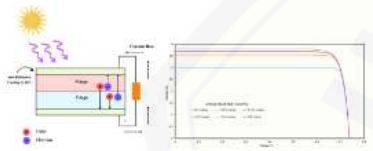

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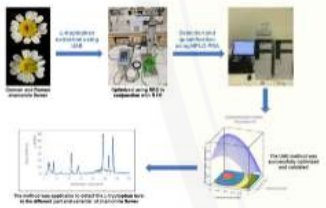

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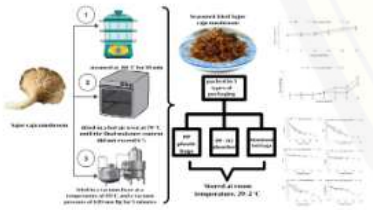

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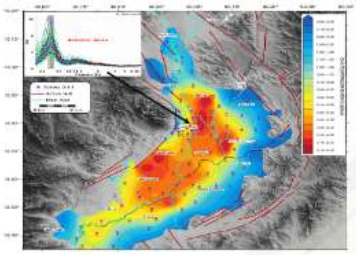

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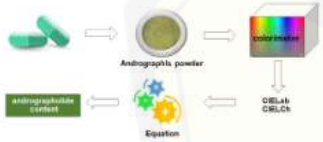

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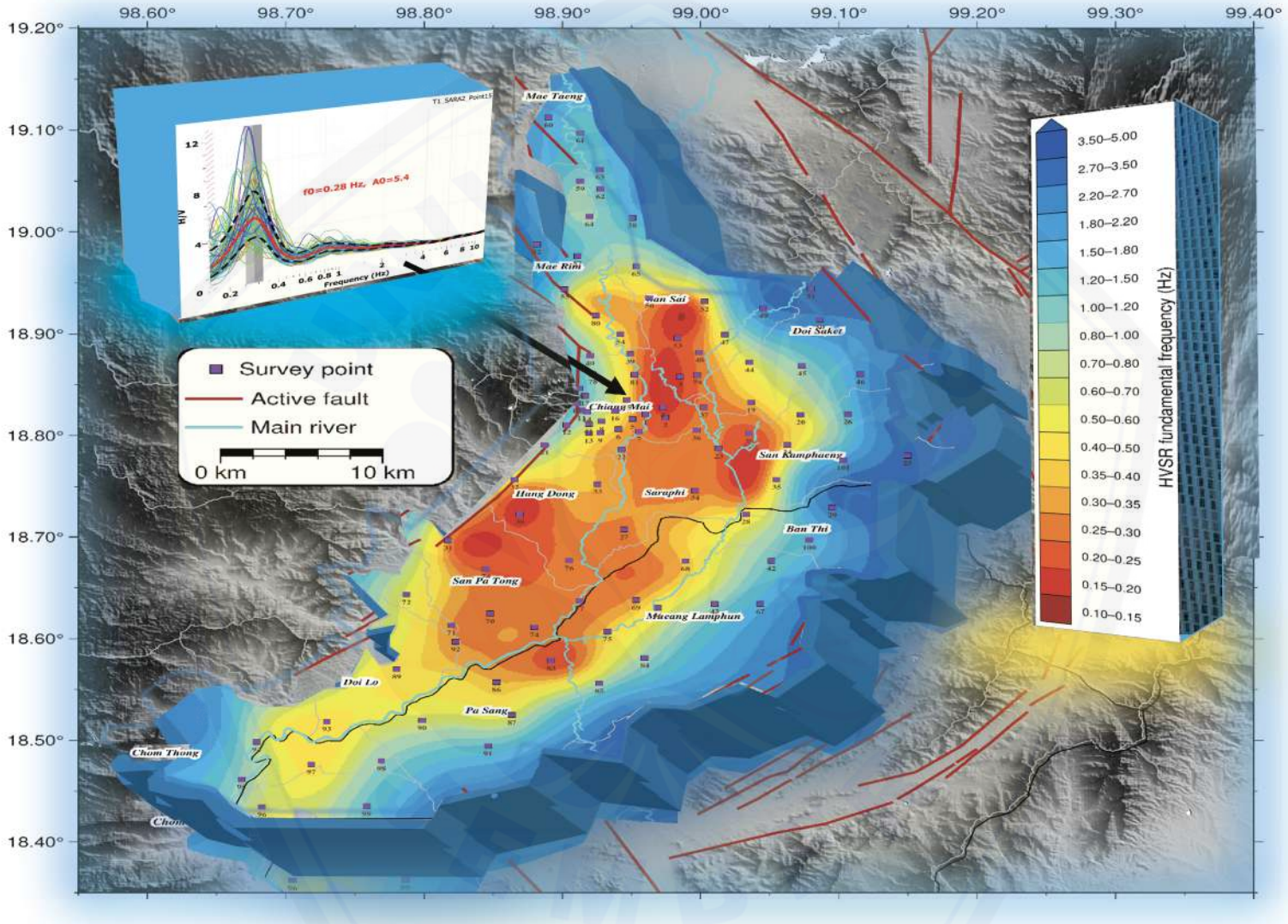

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Antibacterial Activity of Robusta Coffee (*Coffea Canephora)* Husk Extract Against *Enterococcus Faecalis* and *Phorphyromonas Gingivalis*: *In Vitro* Study

Rendra Chriestedy Prasetya[1], Nadie Fatimatuzzahro[1,*], Tantin Ermawati[1], Shinta Kristina[2] and Raden Rara Hanifa Prabaningrum[2]

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Abstract

Enterococcus faecalis is one of the bacteria that is commonly found in infected root canals, necrotic pulp and failure of root canal treatment, while *Phorphyromonas gingivalis* play a role in inducing periodontitis. Both bacteria can enter the blood circulation and lead to risk factors for systemic disease such as atherosclerosis. ChKM is the most widely used root canal sterilization, and also 0.2 % *chlorhexidine* mouthwash for long-term usage. It can destroy a potent cell and is allergenic. Developing an alternative material for root canal sterilization and mouthwash for plaque control is necessary. One of the alternative natural products that is predicted to have antibacterial effect is robusta coffee husk peels. This research aimed to investigate the antibacterial activity of robusta coffee (*Coffea canephora*) husk extract on *E. faecalis* and *P. gingivalis.* The robusta coffee husk was extracted using the maceration process with 96 % ethanol as the solvent. This research is an experimental laboratory, was tested by disk diffusion methods (*Kirby-Baurer*) using Gram-positive *E. faecalis* ATCC 29212 and Gram-negative *P. gingivalis* ATCC 33277 consisted of 6 groups: Aquadest as a negative control group, ChKM (for *E.facecalis*) and 0.2 % chlorhexidine (for *P. gingivalis*) as positive control, 4 treatment groups of coffee robusta husk extract with different concentrations there were 250, 250, 500, 750 and 1,000 mg/mL. The antibacterial effect was examined by measuring the clear zone around the disk paper. The results of this study proved the presence of antibacterial activity of coffee robusta husk extract against *E. faecalis* and *P. gingivalis*. The higher concentrations followed with a greater antibacterial effect.

Keywords: *Enterococcus faecalis*, *Phorphyromonas gingivalis*, Antibacterial, Robusta coffee husk extract, Infected root canals, Periodontitis

Introduction

Enterococcus faecalis is a facultative anaerobic bacteria commonly found in root canal-treated teeth with necrotic pulp and may cause endodontic treatment failure [1]. The prevalence range of *E. faecalis* in endodontic infections was found to be high, valued at 40 % in primary endodontic infections and 22 - 77 % in persistent infections [2]. Currently, the bacteria have been found resistant to various intracanal medicaments [3]. *E. faecalis* is known to be able to enter the dentinal tubes to form biofilms and survive in high pH for a long enough time at a state of little nutrient intake. In addition, the bacteria could compete with other microorganisms and had extraordinary virulence, such as lytic enzymes, gelatinase, cytolysin, aggregation substance, pheromones, and lipoteichoic acid, allowing it to stay in the root canal despite the treatment performed. *E. Faecalis* can also cause root canal infection to progress to the periapical tissues which result in the onset of periapical abscess and enter the blood circulation [2,4].

Periodontal disease is the most common disease with a prevalence of 74.1 % in Indonesia [5]. Periodontal disease is a chronic inflammatory disease that causes inflammation by bacteria in the periodontium. *Porphyromonas gingivalis* is one of the microorganisms that act as a keystone pathogen in periodontal disease [6]. *P. gingivalis* is a Gram-negative and its virulence factors, such as capsule, fimbriae, gingipains, and lipopolysaccharide (LPS) play a role in inducing periodontitis [7]. Some studies suspect this bacterial invasion causes intravascular infection can spread its products throughout the body and become a factor risk for systemic disorders, such as cardiovascular disease (CVD), atherosclerosis, stroke, and premature birth [6].

It is important to administer intracanal medicament to eliminate *E. Faecalis* in the root canal [4] and plaque control material for eliminating *P. gingivalis*. One of common medicaments to sterilize the root canal is ChKM (C*hlorophenol Kamfer Menthol)* which has disinfectant properties and broad spectrum. ChKM can quickly diffuse in dentinal tubules and destroy the bacteria membrane by binding on its proteins and lipids. However, its disadvantages relate to it killing potential cells and is allergenic, triggering adverse immune response [8]. Treatment efforts for periodontal disease are done by scaling and root planning and using mouthwash, namely chlorhexidine. 0.2 % *chlorhexidine* mouthwash for long-term usage has side effects in the form of dental discoloration and mucositis [9].

Coffee is the largest agricultural commodity in the world, especially robusta coffee. Robusta coffee is widely grown in Jember City, Indonesia [10]. In coffee processing 40 - 50 % of coffee husks are produced, but they are only considered as waste, animal feed and, fertilizer in plantations. Robusta coffee husk extract is one of the natural components that is empirically utilised as an antibacterial [11]. Coffee has been known as a potential phitopharmacy plant [12]. A prior study showed that robusta coffee husk peels contained secondary metabolite compounds named polyphenols with antibacterial properties [13]. Robusta coffee husk peels contain active compounds of flavonoids, tannins, saponins, and alkaloids that exhibit antibacterial activity [14,15]. Other studies also identified the presence of antibacterial properties of robusta coffee husk extract against *Streptococcus mutans* and *Lactobacillus acidophilus* [16]. This research aimed to investigate the antibacterial activity of robusta coffee *(Coffea canephora) husk* extract against *E. faecalis* and *P. gingivalis.*

Materials and methods

Ethical approval for antibacterial assay (No.1382/UN25.8/KEPK/DL/2021) was provided by the Ethical Committee of Medical Research at the Faculty of Dentistry at Jember University. Antibacterial assay was divided into 6 groups, namely: Robusta coffee extract concentrations of 250, 500, 750, 1,000 mg/mL, positive control (CHKM and 0.2 % chlorhexidine), and negative control (Aquades sterile). List of tools and materials are: Blender (Philips, Holland), spatula (Pyrex, Japan), filter paper (Whatman), digital scales (Scale SF400, China), test tube (Iwaki Pyrex, Japan), rotary evaporator (Heidolph, Germany), vortex (Labinco L46, Netherlands), petridish (Iwaki Pyrex, Japan), oven (Binder, Germany), laminar air flow (Labtech, Indonesia), incubator (LabTech, Indonesia), autoclaf (Memmert, Germany), Erlenmeyer tube (Schott Duran, Germany), spectrofotometer (Boeco S-22, Germany), micropipet (Socorex Acura 825, Swiss), digital caliper (Krisbow, Indonesia), Ose neddle (Nikrom, Indonesia), gelas ukur (Iwaki Pyrex, Japan), Robusta coffee husk extract, S.mutans, P. gingivalis, Mueller Hinton Agar (MHA) (HiMedia, India), Mueller Hinton Broth (MHB) (HiMedia, India), aquadest (Onelab, Indonesia), etanol 96 % (No Brand), chlorhexidine 0.2 % (Minosep Minorock, Indonesia), paper disc (Oxoid, United Kingdom).

Preparation of robusta coffee husk extract

The maceration process of robusta coffee husks was washed and dried in the sun and put in an oven for 24 h at 50 °C. The rind of dried and crushed robusta coffee fruit is weighed to 800 g are put in 96 % ethanol solution with a ratio of 1:5 for 3 days. The maceration results were filtered through fine filter paper and evaporated with a rotary evaporator for 12 h. The preparation is obtained by mixing robusta coffee husk extract according to the weight of the concentration in a test tube containing 1 mL of aquades steril and homogenising it with a vortex.

Inoculum suspension

E. faecalis ATCC 29212 and *P. gingivalis* ATCC 33277 were the microorganisms used. One ose of bacterial culture was placed in a tube containing 2 mL of Muller Hinton Broth (MHB) liquid medium, and the suspension was vibrated using a vortex and adjusted for turbidity to the 0.5 McFarland standard.

Antibacterial assay

The disc diffusion method used in this study for the antibacterial test (*Kirby-Bauer*). Each suspension of *E. faecalis* was streaked (zig-zag scratched) onto the surface of Muller Hilton Agar (MHA) and *P. gingivalis* into the surface of blood agar media with a cotton swab. Each group's solutions were poured onto disc paper using a micropipette and left to infuse. The disc paper was placed on the surface of inoculated media, then the petridish was closed and placed in an incubator at 37 °C for 24 h. The restricted growth seen in the clear zone surrounding the paper disc, was measured using a vernier caliper. The diameter of the circle was used to calculate the circular inhibition zone, while the long and short diameters were added together and divided by 2.

To get the elliptical inhibition zone, if the inhibition zone did not appear on the paper disc, the inhibition zone would be 0.00 mm [17].

Statistical analysis

Statistical tests were performed using SPSS 2.6 at a significance level of 95 %. One-way Anova parametric test was employed for all treatment groups, followed by the post Hoc LSD test to determine the significant differences in each group ($p < 0.05$) [18].

Results and discussion

The inhibition zone exhibited on the paper disc varied among robusta coffee husk extract groups. The biggest to the smallest inhibition zone displayed on the paper disc with robusta coffee husk extract were from 1,000 to 750 to 500 and to 250 mg/mL. The higher level of the concentration, the bigger the inhibition zone appeared. The *p* value for the one-way Anova and post Hoc LSD tests was 0.000, indicating that is a significant difference in the average diameter of the inhibition zone across groups.

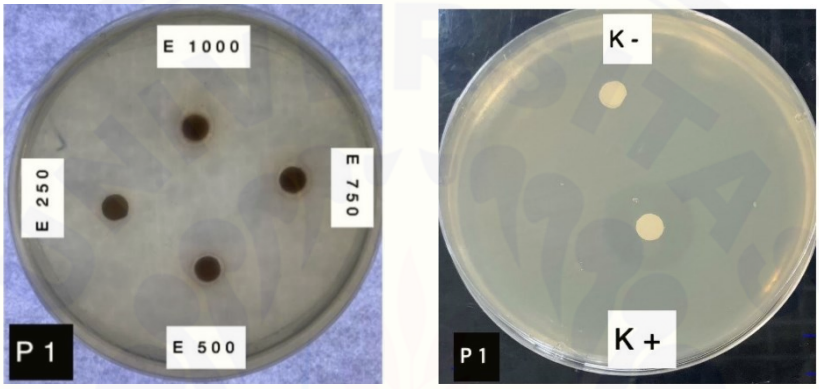

Figure 1 A) Inhibition zone of various robusta coffee husk extract against *E. faecalis s*; B) Inhibition zone of positive (K+) and negative controlled-groups (K−), E250: 250 mg/mL extract concentration; E500: 500 mg/mL extract concentration; E750: 750 mg/mL extract concentration; E1,000: 1,000 mg/mL extract concentration.

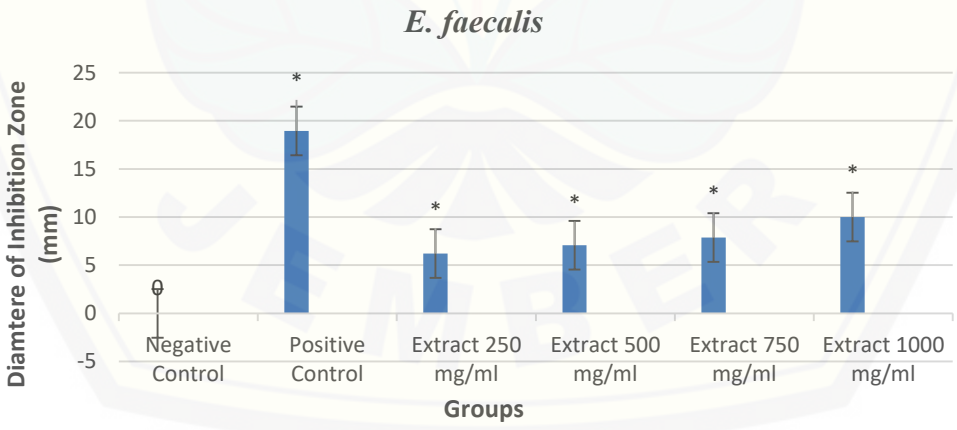

Figure 2 Histogram average score of inhibition zone diameter (mm) of robusta coffee husk extract against *E. faecalis* ($^{*}p < 0.05$).

The antibacterial activity of robusta coffee husk extract was classified based on the diameter of the inhibition zone that appeared. Antibacterial activity was classified into 4 levels. The antibacterial activity was considered low if the diameter was ≤ 5 mm, medium with the diameter was 6 - 10 mm, strong with the diameter was 11 - 20, and superior with the diameter was ≥ 21 mm. All concentrations of robusta coffee husk extract had medium antibacterial activity.

The results showed the antibacterial activity of robusta coffee husk extract against *P. gingivalis* (**Figure 3**). An inhibition zone was formed by robusta coffee husk extract and the positive control (0.2 % *chlorhexidine*), negative control showed no inhibition zone (0 mm).

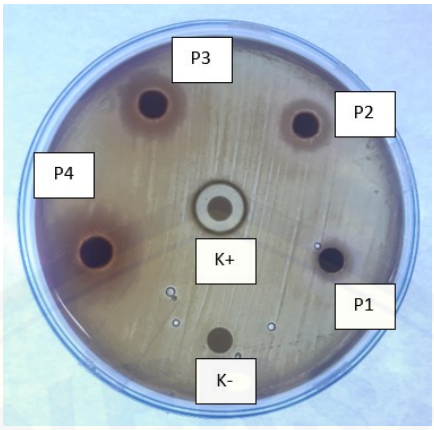

Figure 3 Antibacterial activity against *P. gingivalis*. P1: 250 mg/mL extract concentration; P2: 500 mg/mL extract concentration; P3: 750 mg/mL extract concentration; P4: 1,000 mg/mL extract concentration; K+: Positive control; K−: Negative control.

Robusta coffee husk extract has proven antibacterial inhibition against *P. gingivalis*. The visible inhibition zone is a clear area around the disc paper, but it has dark brown due to the brown robusta coffee extract solution, so the brown dye diffuses around the disc paper. In addition, the presence of tannins in the skin of robusta coffee husk functions as a substance that gives a brownish color pigment [19,20].

The results of the inhibitory zone formed in antibacterial tests of *P. gingivalis* can be divided into, the radical zone (radical zone) and the iradical zone (iradical zone). The radical zone or also called the kill zone is indicated by the presence of a clear zone around the paper disc where in this area no bacterial growth is found at all. The iradical zone is a zone located between the bacterial colony area and the clear zone where there is still bacterial growth, characterized by areas that appear infertile or cloudier when compared to negative controls. The iradical zone is also called the inhibitory zone because in this zone bacterial growth is only inhibited but not killed [21].

The average of the inhibition zone revealed that the robusta coffee husk extract at 1,000 mg/mL resulted in the biggest inhibition zone against *P. gingivalis* of 20.07 (**Figure 4**).

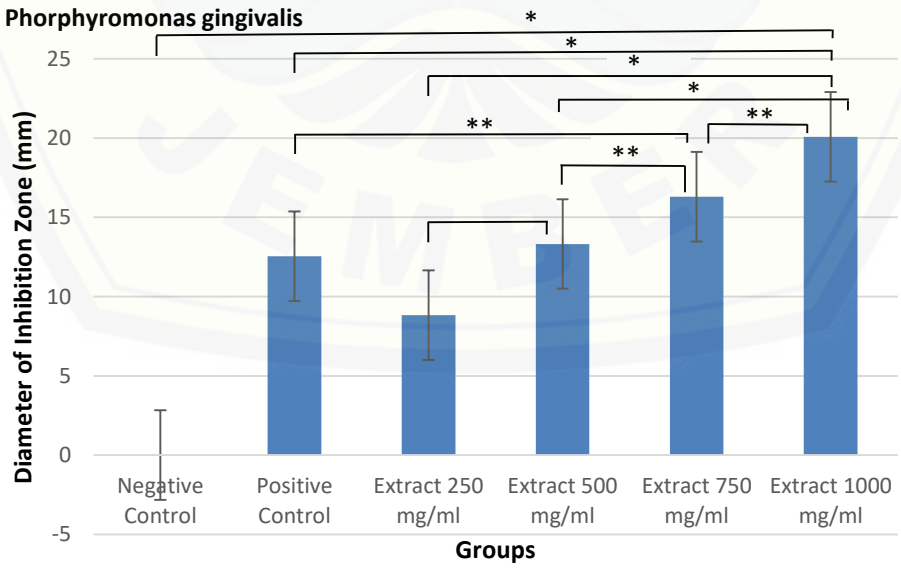

Figure 4 Average inhibition zone diameter against P. gingivalis ($^{*}p < 0.05$, $^{**}p > 0.05$).

The active chemicals found in robusta coffee husk extract are assumed to be responsible for an antibacterial. Its mechanism related to the function of secondary metabolites substances contained in robusta coffee husk extract was proven to have antibacterial activity [11]. The extract has been known to have secondary metabolite compounds such as polyphenols, flavonoids, tannins, saponins, and alkaloids [14,22].

The action mechanism of flavonoids as positive gram antibacterial agents like *E. faacalis*, is related to its structure which has aromatic rings, damaging the cytoplasmic membrane. The damage allows nucleotide and amino acid to leave cells. By the time of cytoplasmic membrane is damaged, ion H+ from flavonoid would attack polar group (phosphate group), hence phospholipid molecule would be unravelled. This leads to inactivity of the phospholipid to preserve the form of the cytoplasmic membrane, thus the cytoplasmic membrane would be leaked [23,24].

Flavonoids which are one of the antibacterial compounds contained in extracts have the ability as bacteriostatic and bactericidal, by inhibiting the synthesis of bacterial cell walls, inhibiting bacterial toxins and damaging phospholipids in the bacterial cytoplasmic membrane [23]. Flavonoids as an antibacterial property by interfering the cell wall biosynthesis of *P. gingivalis* by inhibiting fatty acid synthase type-II (FAS-II) from lipopolysaccharide which is an important component for bacterial synthesis, so that the cell wall will be damaged and flavonoids will enter the cell nucleus and contact with bacterial DNA causing bacterial lysis and death [25]. Flavonoids can also inhibit pili and flagellum so that adhesion and colonization of bacteria do not occur [26].

Alkaloid is contained in robusta coffee husk extract also acts as an antibacterial agent. It is a basic nitrogen heterocyclic compound. The action mechanism of the compound against *E. faecalis* is connected to its basic group that reacts with acid compound in the bacteria, which is the DNA. This leads to protein synthesis and the nucleate acid of *E. faecalis* agitated [27]. Alkaloids interfere with cell wall synthesis by penetrating LPS from *P. gingivalis*. Alkaloids interferec with peptidoglycan formation and damage cell walls. As a result of the rupture of the cell wall, alkaloids that are alkaline will interacts with acidic compounds in DNA and interfere with enzymes topoisomerase, so bacteria cannot multiply and causing decrease in the number of bacteria [28].

It is also proven that the extract contains the other active compound, which is polyphenol. Bacteria's cell wall plays a significant role in osmotic protection. However, polyphenol can damage it, which results in lowering the cell's tolerance to high ionic strength and low osmotic pressure. The damaged cell wall allows polyphenol to interact with the bacteria's plasma membrane and leads to lowering negative charge of the bacteria, change of hydrophobicity, and occurrence of local rupture that make the cell leaked and release cellular molecules. Polyphenol is also known for its ability to agitate the positive gram bacterial quorum sensing system which is essential in forming biofilm. Due to the presence of polyphenol, the system is agitated and the bacteria's survival ability decreases for skipping the biofilm formation [29].

Polyphenol molecules are able to reduce the length and density of fimbriae. Furthermore, polyphenols will bind to lipid-A from lipopolysaccharides (LPS) in *P. gingivalis*. Polyphenols will damage the bacterial cell wall, thereby lowering the cell's tolerance to high ionic strength and low osmotic pressure. It causes polyphenols to interact with the plasma membrane of bacteria and resulting in a decrease in the negative charge of bacteria, changes in hydrophobicity, the occurrence of local rupture, and the formation of pores so that cell membranes will leak and release cellular molecules [29].

Another compound that acts as an antibacterial agent is tannin. The action mechanism of tannin against *E. faecalis* is by forming hydrogen bonds with the protein of the bacteria cell, thus the protein is denaturized and the bacteria cell's metabolism is agitated. The hydrogen bond formed between tannin and protein would cause molecular shape changes in the protein and reduce the biochemical activity of *E. faecalis*. The reduction of biochemical activity in *E. faecalis* may inhibit reverse transcriptase enzymes and topoisomerase DNA, hence the bacteria cell cannot be formed [30].

Tannins can bind complexes with proteins, polysaccharides, alkaloids, and minerals can cause damage to bacterial cells. Tannins can reduce the length of fimbriae by 60 % in bacteria and can interfere with cell permeability by scrunching the walls cell so that the formation of the cell wall is not complete. Further tannins will enter the cell nucleus and interact with DNA so that enzymes inhibit topoisomerase. Bacterial enzymes are inhibited by absorbing the substrate macronutrient minerals such as zinc that are needed for bacterial growth. Bacteria cannot multiply, the number of bacteria decreases due to inhibition of the topoisomerase enzyme and results in bacterial death [31,32]. A previous study in periodontitis rats model which induced *P. gingivalis* showed the antibacterial activity of cocoa pod extract gel is found in the content of tannins. It has the ability to inhibit the work of protease enzymes which cause bacterial metabolic processes to be disrupted so that they can cause death in bacteria [33].

The other component contain in robusta coffee peel extract is saponin. This active compound which surface looks like detergent can reduce tense of the bacteria cell wall and is also proven to damage *E. faecalis* membrane permeability. The action mechanism of saponin is by diffusing through outer membrane and the cell wall then binding the cytoplasm membrane, which leads to agitating and reducing the cytoplasm membrane and causes cytoplasm to leake and leave the bacteria cell [34].

The efficacy of an antimicrobial compound to inhibit microbial growth depends on the level of concentration, type of microbe, pH level, and dissolved substance or organic matter [35]. Concentration level would affect the diffusion activity to inhibit the growth of *E. Faecalis* and *P. gingivalis*. This study showed that the higher concentration level of the extract, the biggest inhibition zone occurred. It was assumed that higher level of concentration creates more antibacterial active compounds. Hence, the activity to inhibit bacterial growth is more optimal [36,37]. Other research also affirmed that higher concentration test solution is followed by larger inhibition zone [22].

The results showed that ChKM as the positive control for *E. faecalis* has the biggest inhibition zone compared to the groups of robusta coffee husk extract. The average diameter of inhibition zone created by ChKM against *E. faecalis* was 18.95 mm and can be classified as strong antibacterial activity. The antibacterial effect of ChKM results from its main ingredient which is parachlorophenol that can destroy various microorganisms, one of which is *E. faecalis*. ChKM is known as an intracanal medicament that has antibacterial activity with broad spectrum. ChKM has better antibacterial disinfectant compared to other phenolic medicaments (paramonochlorophenol, thymol and cresol) [38]. In addition, according to previous study of all antibacterial agents tested from ChKM, chlorhexidine, polyhexanide, to povidone iodine, it shows that the antibacterial with highest effectiveness to eliminate *E. faecalis* is ChKM. The antibacterial effect of ChKM is based on its activity to destroy the bacteria membrane by binding its protein and lipid [39].

Zero-point-two percent chlorhexidine as a positive control for *P. gingivalis* is an effective antibacterial ingredient in inhibiting and killing bacteria in the oral cavity. Chlorhexidine with a high concentration (> 0.1 %) is charged with cations (+) while bacteria have an anion charge (−), so this can make it easier chlorhexidine to attach to bacteria by penetrating and damaging *P. gingivali*s cells wall that causes cytoplasmic leakage from within the cell and produce effects bactericidal (lysis and death of bacterial cells) [40]. While sterile aquades as a negative control are compounds that are neutral so that no inhibition zone forms around the disc and do not give affect antibacterial growth [41].

Besides the antibacterial agent, the bacteria structure also plays role in the antibacterial action mechanism. Each bacterium has a different composition and structure of cell wall. The sample needs to diffuse into the cell through the bacteria cell wall to kill the bacteria [42,43]. The ability of each bacterium against antibacterial action varies from one to another, depending on the thickness and composition of the cell wall. This bacteria is a kind of positive gram bacteria which cell wall structure has more than 40 % of peptidoglycan [44]. The positive gram bacteria of *E. faecalis* has relatively simple structure of cell wall consisting of 2 layers, that are thick peptidoglycan layer and inner membrane. This makes the *E. faecalis* susceptible to antibacterial agents because the agent can easily diffuse in [45].

P. gingivalis is a Gram-negative bacterium has a complex structure, there are endotoxins in the form of lipopolysaccharides in its walls and the outer membrane surrounds the plasma membrane has peptidoglycan in it. Sensitivity to incoming antibacterial compounds may be hindered due to the complex bacterial cell structure in *P. gingivalis* [46]. This is in line with previous research which shows that Gram-positive bacteria have a larger inhibitory zone compared to Gram-negative bacteria [47]. This is due to the structure of *E. faecalis* in contrast to *P. gingivalis*. *P. gingivalis* is a Gram-negative bacterium has a complex cell wall layer and there are endotoxins in the form of lipopolysaccharides, while *E. faecalis* has a simpler cell wall structure, which is 90 % of its wall consists of peptidoglycan [48]. This is caused by the difference in the inhibitory zone between *E. faecalis* and *P. gingivalis*. The inhibitory zone formed in *E. faecalis* is the radical zone, while in *P gingivalis* an iradical zone is formed.

Conclusions

Robusta coffee *(Coffea canephora)* husk extract at concentrations of 250, 500, 750 and 1,000 mg/mL exhibited antibacterial activities against *E. faecalis* and *P. gingivalis*. It was also found that the higher the concentration, the bigger inhibition zone appearred. Robusta coffee husk extract with a concentration of 1,000 mg/mL showed the greatest inhibition zone.

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References

[1] C Rodríguez-Niklitschek and GHV Oporto. Clinical implications of *Enterococcus faecalis* microbial contamination in root canals of devitalized teeth: Literature review. *Rev. Odontológica Mexic.* 2015; **19**, e177-e182.
[2] V Gopikrishna. *Grossman's endodontic practice.* 14th ed. Wolters Kluwer India Pvt. Ltd., Gurugram Haryana, India, 2021.
[3] T Dammaschke, N Jung, I Harks and E Schafer. The effect of different root canal medicaments on the elimination of *Enterococcus faecalis* ex vivo. *Eur. J. Dent.* 2013; **7**, 442-8.
[4] M Torabinejad, AF Fouad and S Shabahang. *Endodontics principles and practice.* 6th ed. Elsevier, Philadelphia, 2021.
[5] HN Azuro, M Yunus and RW Gayatri. Early detection of periodontitis in public health centres in Malang: An overview. *KnE Life Sci.* 2021; **2021**, 134-48.
[6] MG Newman and HH Takei. Newman and carranza's clinical periodontology. 13th ed. Saunders, London, 2018.
[7] TR Septiwidyati and EW Bachtiar. The role of *porphyromonas gingivalis* virulence factors in periodontitis immunopathogenesis. *Dentika Dent. J.* 2020; **23**, 6-12.
[8] Y Wilda, FM Satryani, S Maemonah and J Christyaningsih. The inhibitory effect of *carica papaya* cv . Thailand leaf extract to the growth of *Enterococcus faecalis in vitro*. *Scholars J. Dent. Sci.* 2017; **4**, 262-6.
[9] D Permatasari, LY Budiarti and ML Apriasari. Antifungal effectiveness of methanol extract of Mauli banana stem (*Musa acuminata*) and chlorhexidine 0.2 % against *Candida albicans*. *Dentino Jurnal Kedokteran Gigi* 2016; **1**, 10-4.
[10] DI Wardhana, E Ruriani and A Nafi. Characteristics of robusta coffee husk obtained from dry processing method of smallholder coffee plantation in east java. *Agritrop* 2019; **17**, 220-9.
[11] H Rante, Subehan, R Wulandari and YM Evary. Antibacterial activity of robusta coffee (*Coffea robusta L.*) peel extract against human pathogenic bacteria. *J. Exp. Biol. Agr. Sci.* 2021; **9**, 264-8.
[12] RC Prasetya, N Fatimatuzzahro and W Jatmiko. Activity of robusta coffee on the expression of CRP and MMP-9 against hyperlipidemia. *Coffee Sci.* 2020; **15**, 6-11.
[13] VA Bonilla-Hermosa, WF Duarte and RF Schwan. Utilization of coffee by-products obtained from semi-washed process for production of value-added compounds. *Bioresource Tech.* 2014; **166**, 142-50.
[14] DA Winahyu, S Marcellia and MI Diatri. Test of antioxidant activity of robusta coffee peel extract (*Coffea canephora*) in cream preparations. *Jurnal Farmasi Malahayati* 2021; **4**, 82-92.
[15] AR Prihadi, A Maimulyanti and B Mellisani. Antioxidant activity, tannin content and dietary fiber from coffee husk extract and potential for nutraceutical. *Rasayan J. Chem.* 2020; **13**, 955-9.
[16] AR Kusumawardani, AM Machbub, RC Prasetya, N Fatimatuzzahro and T Ermawati. Antibacterial activity of robusta coffee (*coffea robusta*) husk extract against *streptococcus mutans* and *lactobacillus acidophilus*: *In vitro* study. *J. Orofacial Sci.* 2022; **14**, 88-92.
[17] J Hudzicki. *Kirby-bauer disk diffusion susceptibility test protocol*. American Society for Microbiology, Washington DC, 2016.
[18] E Tyastirin and I Hidayati. *Parametric statistics for health research*. Architecture study program at UIN Sunan Ampel, Surabaya, Indonesian, 2017.
[19] RA Indriana, P Astuti and A Kurniawati. Uji daya hambat ekstrak metanol daun ungu (Graptophillum pictum (L.) Griff) terhadap pertumbuhan bakteri saluran akar gigi (Inhibition test of purple leaf (graptophillum pictum (L.) griff) methanol extract toward root canal bacteria's growth). *Pustaka Kesehatan* 2017; **5**, 145-50.
[20] RT Yuliani and R Priambodo. Characteristics of cascara types and fermentation periods to kombucha cascara characteristics. *Jurnal Pro Stek* 2021; **3**, 87-97.
[21] PO Samirana, YB Murti, RI Jennie and EP Setyowati. Antibacterial and cytotoxic activities of supernatant and mycelium extracts from fermentation of fungal symbiont *Trichoderma reesei* TV221. *J. Appl. Pharmaceut. Sci.* 2021; **11**, 90-9.
[22] MR Harahap. Inhibitory power activity of robusta coffee fruit meat waste (*coffea robusta L.*) aceh against *S.aureus* and *E.coli*. *Jurnal Kesehatan* 2018; **9**, 93.

[23] X Yixi, W Yang, F Tang, X Chen and L Ren. Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. *Curr. Med. Chem.* 2015; **22**, 132-49.
[24] G Yuan, Y Guan, H Yi, S Lai, Y Sun and S Cao. Antibacterial activity and mechanism of plant flavonoids to Gram-positive bacteria predicted from their lipophilicities. *Sci. Rep.* 2021; **11**, 1-15.
[25] A Biharee, A Sharma, A Kumar and V Jaitak. Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. *Fitoterapia* 2020; **146**, 1-22.
[26] L Qomariyah, FUA Panjaitan and R Adhani. Antibacterial effectivity of flavonoid fraction of ramania leaf extract (*Bouea macrophylla Griffith*) against *Porphyromonas gingivalis*. *Dentino Jurnal Kedokteran Gigi* 2021; **6**, 72-8.
[27] Z Suhaili, CC Yeo, HN Yasin, NA Badaludin and ZA Zakaria. Antibacterial profile of Jatropha curcas latex extracts against selected human pathogenic bacteria. *African J. Infect. Dis. Res.* 2018; **5**, 1-8.
[28] J Sebastian and AS Widyarman. Roselle flower petals extract inhibits periodontal pathogenic biofilms. *J. Dentomaxillofacial Sci.* 2021; **6**, 102-5.
[29] C Papuc, GV Goran, CN Predescu, V Nicorescu and G Stefan. Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: Classification, structures, sources, and action mechanisms. *Compr. Rev. Food Sci. Food Saf.* 2017; **16**, 1243-68.
[30] I Nirwana, D Rianti, RH Soekartono, RD Listyorini and DP Basuki. Antibacterial activity of fig leaf (*Ficus carica Linn*.) extract against *Enterococcus faecalis* and its cytotoxicity effects on fibroblast cells. *Vet. World* 2018; **11**, 342-7.
[31] M Girard and G Bee. Invited review: Tannins as a potential alternative to antibiotics to prevent coliform diarrhea in weaned pigs. *Animal* 2019; **14**, 95-107.
[32] DK Khotimah, IWAK Firdaus and ML Apriasari. The inhibitory activity of kelakai leaf extract against the growth of Porphyromonas gingivalis ATCC® 33277™. *Dentino Jurnal Kedokteran Gigi* 2020; **5**, 104-9.
[33] YC Rahayu, EM Setiawatie, RP Rahayu, NN Sakinah, B Kusumawardani, A Gunadi and PH Ryandhana. Effects of cocoa pod husk extract (*Theobroma cacao L.*) on alveolar bone in experimental periodontitis rats. *Trends Sci.* 2023; **20**, 6535.
[34] M Arabski, A Węgierek-Ciuk, G Czerwonka, A Lankoff and W Kaca. Effects of saponins against clinical *E . coli* strains and eukaryotic cell line. *J. Biomed. Biotechnol.* 2012; **2012**, 286216.
[35] N Tria, F Ermawati, T Fatmawati and DW Aju. Antibacterial activity of apple peel (*malus sylvestris mill*.) variety of manalagi extract against the growth of *streptococcus viridans*. *Jurnal Kedokteran Gigi Universitas Jember* 2014; **11**, 23-6.
[36] AB Utami, IG Sudarmanto and IW Merta. Differences in *staphylococcus aureus* growth inhibition zones at various concentrations of bitter melon's leaf juice *in vitro*. *Meditory* 2015; **1**, 1-5.
[37] R Haghgoo, M Mehran, E Afshari, HF Zadeh and M Ahmad. Antibacterial effects of different concentrations of althaea officinalis root extract versus 0.2 % chlorhexidine and penicillin on *streptococcus mutans* and *lactobacillus* (*In vitro*). *J. Int. Soc. Prev. Community Dent.* 2017; **7**, 180-5.
[38] L Rochyani. The inhibition of leaf extract moringaoleifera on the formation biofilm bacteria *Enterococcus faecalis*. *Denta Jurnal Kedokteran Gigi* 2019; **14**, 44-50.
[39] R Tarigan and T Gita. *Perawatan pulpa gigi (endodonti)*. 3rd ed. Penerbit Buku Kedokteran EGC, Jakarta, Indonesia, 2019.
[40] ZLS Brookes, R Bescos, LA Belfield, K Ali and A Roberts. Current uses of chlorhexidine for management of oral disease: A narrative review. *J. Dent.* 2020; **103**, 103497.
[41] R Sidharta, V Sutanti, D Diah and AN Santi. Effectiveness of hyacinth leaf extract (*Eichhornia crassipes*) against viability of *Porphyromonas gingivalis in vitro*. *E Prodenta J. Dent.* 2021; **5**, 403-13.
[42] GMD Tejada, S Sánchez-gómez, I Kowalski and Y Kaconis. Bacterial cell wall compounds as promising targets of antimicrobial agents I. Antimicrobial peptides and lipopolyamines. *Curr. Drug Targets* 2013; **13**, 1121-30.
[43] L Panawala. Difference between gram positive and gram negative bacteria, Available at: http://pediaa.com/difference-between-gram-positive-and-gram-negative-bacteria/, accessed April 2017.
[44] HD Kusumaningrum, S Nasution, E Kusumaningtyas and DN Faridah. Lysozyme from chicken egg white as an antibacterial agent. *Indonesian Bull. Anim. Vet. Sci.* 2019; **28**, 175.
[45] Y Zhang, J Su and D Wu. *Physiology and pathology of multidrug-resistant bacteria: Antibodies- and vaccines-based pathogen-specific targeting. In*: N Rezaei (Ed.). Physiology and pathology of immunology. InTechOpen, London, 2017.
[46] B Muchtaromah, ES Safitri, PD Fitriasari and J Istiwandhani. Antibacterial activities of curcuma manga Val. extract in some solvents to *Staphylococcus aureus* and *Escherichia coli. In*: Proceedings

of the International Conference on Life Sciences and Technology, Malang, Indonesia. 2020.

[47] D Sylvana, M Amir, CB Purnamasari, A Iskandar and V Asfirizal. Antibacterial activity of ethanol extract of Beluntas leaves on *Streptococcus mutans, Porphyromonas gingivalis* and *Enterococcus faecalis*. *Padjadjaran J. Dent.* 2021; **33**, 191-8.

[48] AA Nurbianti, Alhawaris and S Yani. Inhibition of ethanol extract of yellow root stem (*Arcangelisia flava (L) Merr*) against the growth of *Streptococcus mutans, Porphyromonas gingivalis* and *Enterococcus faecalis*. *Mulawarman Dent. J.* 2021; **1**, 76-89.