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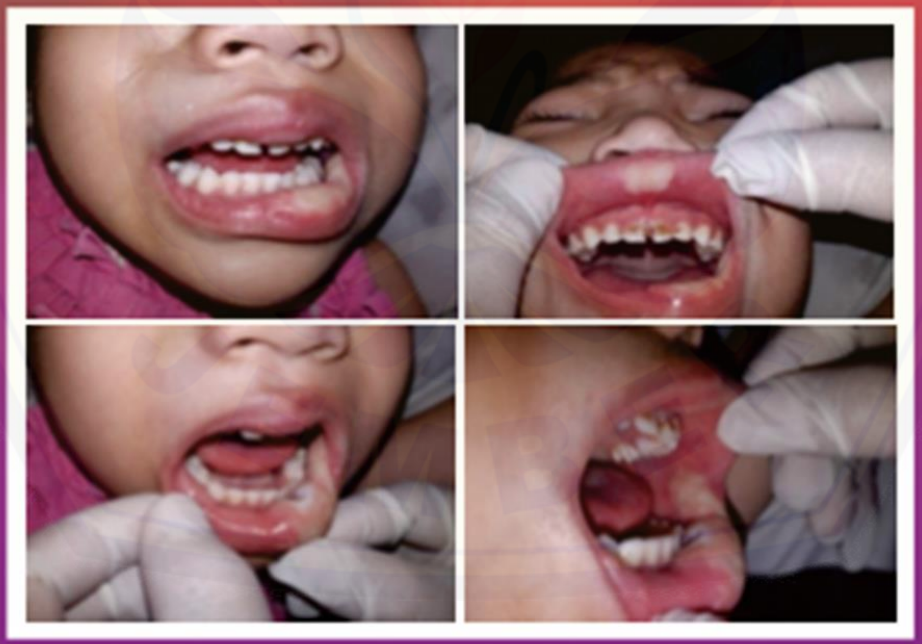

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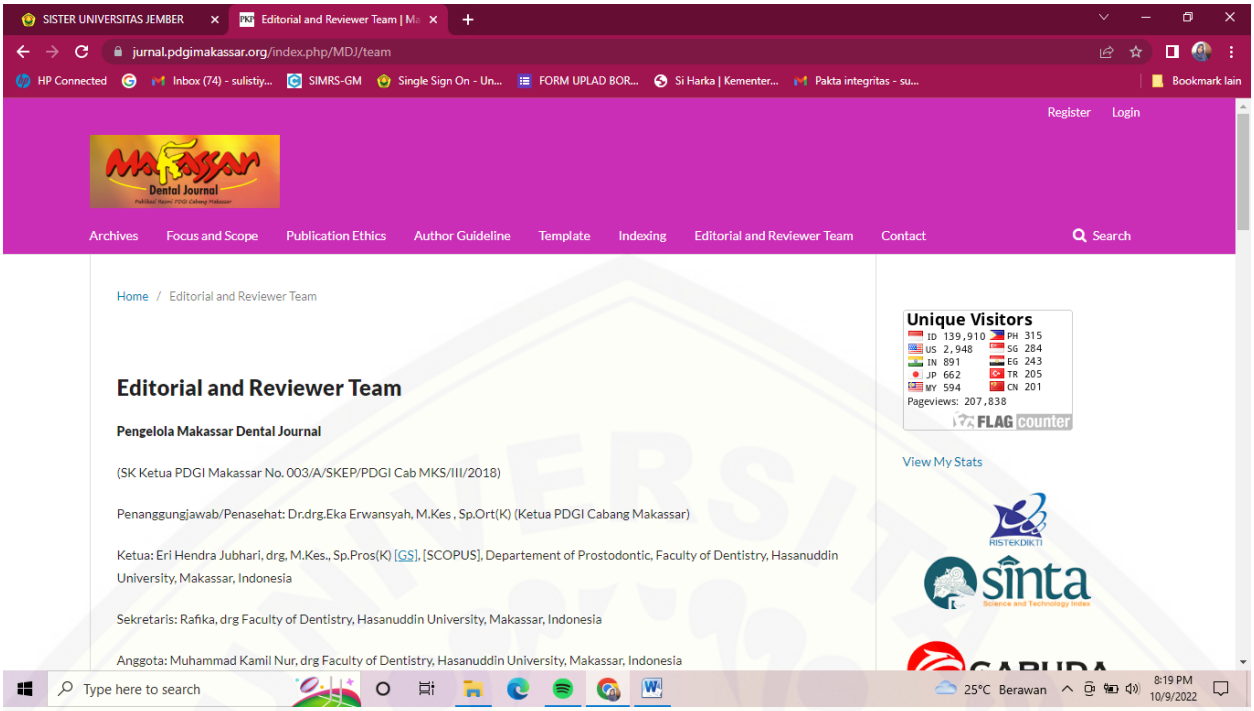

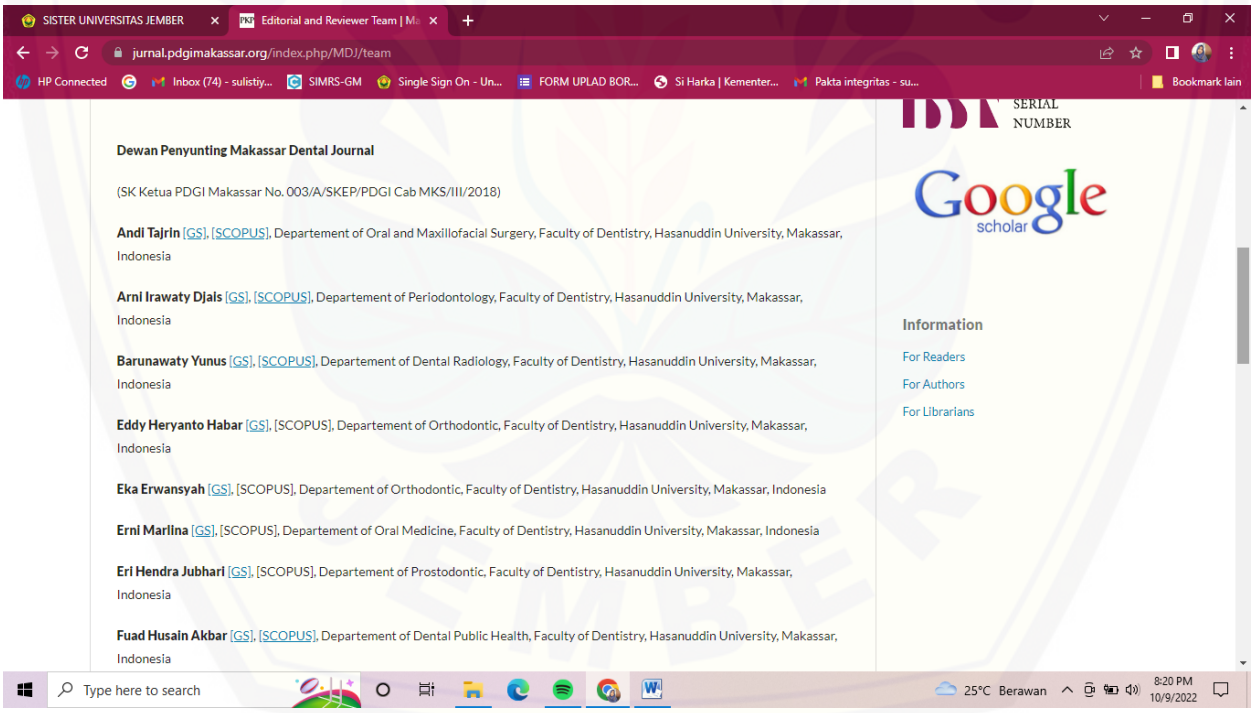

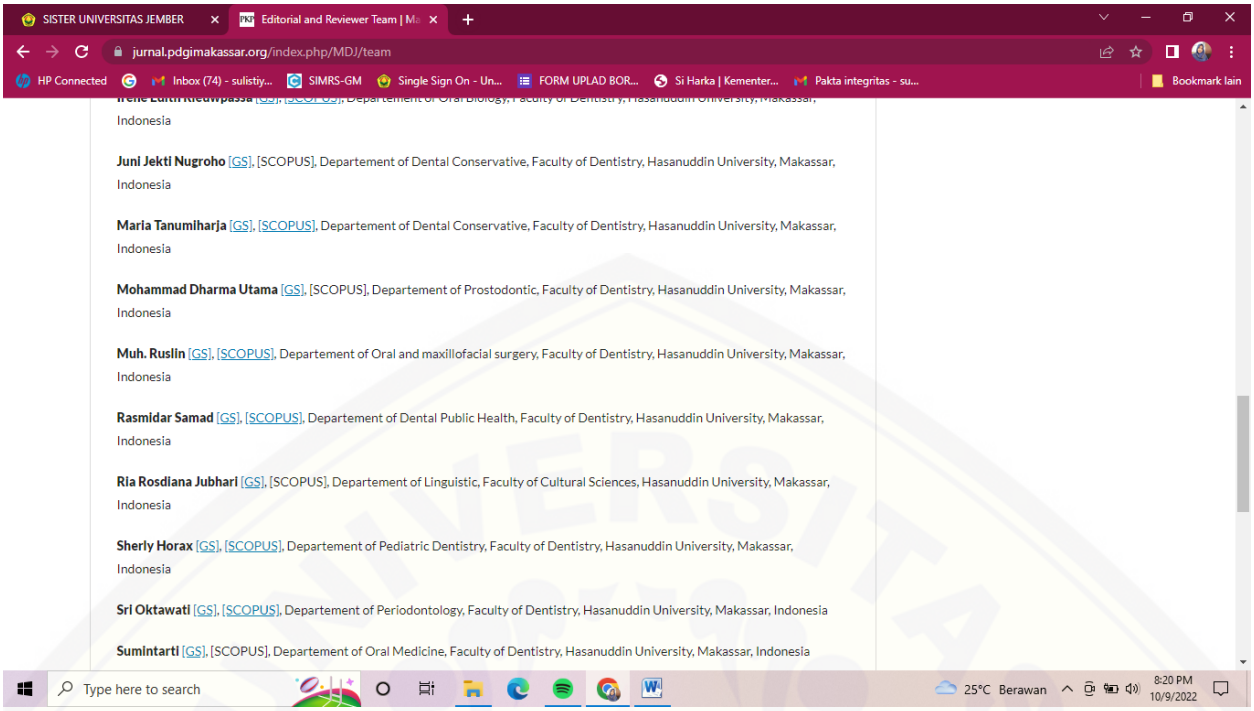

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Daftar Isi

Catatan: Bagi para calon penulis naskah ilmiah dapat mengikuti petunjuk pagi penulis pada halaman terakhir setiap terbitan. Opini dan tulisan sejenisnya dapat diterima dengan syarat tidak mengganggu ketertiban umum dan diketahui kebenarannya oleh Ketua Cabang/Pengwil-nya

Inhibition of green okra (*Abelmoschus esculentus*) extract against *Enterococcus faecalis* in tooth root canals

Penghambatan ekstrak okra hijau (*Abelmoschus esculentus*) terhadap *Enterococcus faecalis* di saluran akar gigi

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ABSTRACT
Enterococcus faecalis is a bacterium that is often found in root canals of teeth and can survive in them even after treatment. The use of 2.5% NaOCl as a root canal irrigation material has disadvantages; toxic and irritating, so other alternatives are needed. Green okra extract contains flavonoids, alkaloids, saponins, tannins, and terpenoids which have antibacterial properties. This study aims to determine the inhibition of green okra extract against *E.faecalis*. It was done experimental laboratory research with a posttest-only control group design. The inhibition test used the disc diffusion method which consisted of 5 research groups, i.e. green okra extract with concentrations of 1.563%, 3.125%, 6.25%, 12.5%, and 2.5% NaOCl. Data were analyzed using non-parametric statistical tests Kruskal Wallis and Mann Whitney. The average of the inhibition zone diameter of the green okra extract concentrations were 1.563% (0 mm), 3.125% (0 mm), 6.25% (15.9 mm), 12.5% (18.03 mm), and 2.5% NaOCl (24.07 mm). The Kruskal Wallis test showed a significance value of 0.000 ($p<0.05$) which meant that there was a difference in the research group, followed by the Mann Whitney test which showed there were significant differences between all research groups except between green okra extract concentrations of 1.563% and 3.125%. It was concluded that green okra extract had an inhibitory effect on the growth of *E.faecalis* at concentrations of 6.25% and 12.5%.
Keywords: *Enterococcus faecalis*, green okra, inhibition

ABSTRAK
Enterococcus faecalis merupakan bakteri yang sering ditemukan dalam saluran akar gigi dan dapat bertahan di dalamnya meskipun telah dilakukan perawatan. Penggunaan NaOCl 2,5% sebagai bahan irigasi saluran akar memiliki kekurangan, yaitu toksik dan iritatif sehingga diperlukan bahan lain. Ekstrak buah okra hijau memiliki kandungan zat aktif flavonoid, alkaloid, saponin, tanin, dan terpenoid yang bersifat antibakteri. Penelitian ini bertujuan untuk mengetahui daya hambat ekstrak buah okra hijau terhadap *E.faecalis*. Dilakukan penelitian eksperimen laboratorium dengan rancangan penelitian *posttest-only control group design*. Uji daya hambat menggunakan metode difusi cakram yang terdiri atas 5 kelompok yaitu ekstrak buah okra hijau konsentrasi 1,563%, 3,125%, 6,25%, 12,5%, dan NaOCl 2,5%. Hasil penelitian dianalisis menggunakan uji statistik non parametrik *Kruskal Wallis* dan *Mann Whitney*. Data nilai rata-rata diameter zona hambat kelompok ekstrak buah okra konsentrasi 1,563% (0 mm), 3,125% (0 mm), 6,25% (15,9 mm), 12,5% (18,03 mm), dan NaOCl 2,5% (24,07 mm). Uji *Kruskal Wallis* menunjukan nilai signifikansi 0,000 ($p<0,05$) yang berarti terdapat perbedaan pada kelompok penelitian; uji *Mann Whitney* yang menunjukkan terdapat perbedaan signifikan antar semua kelompok perlakuan kecuali antara ekstrak buah okra 1,563% dan 3,125%. Disimpulkan bahwa ekstrak buah okra hijau memiliki daya hambat terhadap pertumbuhan *E.faecalis* pada konsentrasi 6,25% dan 12,5%.
Kata kunci: *Enterococcus faecalis*, okra hijau, daya hambat
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INTRODUCTION

Pulp necrosis is a condition following untreated pulpitis. Bacteria, trauma, and some chemical irritants can cause necrosis of the pulp.[1] Microorganism has an important role in infection that causes necrosis of the dental pulp. The results of bacterial culture research on necrotic root canals showed that 57,14% were facultative anaerobic bacteria and 42.86% were the aerobic.[2]

Enterococcus faecalis is a gram-positive facultative anaerobic bacterium that is often found in tooth root canals and can persist in them despite treatment. The prevalence of root canal infections caused by *E.faecalis* ranged 24-77%.[3] Based on Suryandari, et al it is known that 20 out of 30 cases of endodontic infection that had root canal treatment were caused by *E.faecalis*.[4] These bacteria can compete with other microorganisms to enter the dentinal tubules and can survive in low nutriational conditions.[3]

Root canal treatment aims to clean the root canal thoroughly from infected pulp tissue so that the root canal space can be formed and prepared to be filled with a root canal filling material to prevent or minimize reinfection. One of the important steps in supporting the success of root canal treatment is root canal irrigation which aims to facilitate the removal of necrotic tissue, debris, and kill microorganisms from infected root ca-

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nals including *E.faecalis* bacteria.[1]

One of the root canal irrigants that is often used today is 2.5% NaOCl because of its ability to dissolve necrotic pulp tissue, rinse debris out of the root canal, and have broad-spectrum antimicrobial properties. However, the use of 2.5% NaOCl also has disadvantages, i.e. unpleasant odor and taste, irritating when pushed into the periapical tissue, and unable to dissolve inorganic ganic components of the smear layer.[1]

The use of natural plants as medicinal ingredients is widely carried out because they are considered safer and have minimal side effects compared to chemical substances. One of the natural plants that can be used as an alternative to root canal irrigation is green okra (*Abelmoschus esculentus*) that is a tropical plant that is commonly consumed by people. Okra plants widely cultivated in Indonesia to be used as vegetables and medicinal ingredients.[6] Green okra contains various important nutrients and phytochemicals and has several biological activities such as antioxidant, antidiabetic and antibacterial. The results of the phytochemical test of green okra extract obtained active compounds that have the potential as antibacterials such as alkaloids, terpenoids, flavonoids, saponins, and tannins.[7]

Based on Yuliati et al's research, it was known that green okra extract was able to inhibit the growth of *Porphyromonas gingivalis* bacteria with a minimum inhibitory concentration (MIC) at a concentration 3.125% and a minimum bactericidal concentration (MBC) at a concentration of 6.25%.[8] A similar study by Luthfi et al stated that green okra extract was effective in inhibiting the growth of *Aggregatibacter actinomycestemcomitans* (*Aa*) with MIC of 3.125% and MBC of 6.25%.[9] Therefore, the researcher wanted to know the inhibitory power of green okra extract at concentrations of 1.563%, 3.125%, 6.25%, and 12.5% on the growth of *E.faecalis* when compared to 2.5% NaOCl.

METHODS

In experimental laboratory with a posttest-only control group design, the green okra samples were obtained from PT. Mitratani Dua Tujuh, Jember Regency. The plant identification was carried out in Plant Laboraratory, Jember State Polytechnic. Green okra extract was obtained by the maceration method which was carried out at the Bioscience Laboratory of Dental Hospital Jember University. Inhibition research was conducted at the Research Center of the Faculty of Dentistry, Airlangga University. The sample groups were green okra extract with concentrations of 1.563%, 3.125%, 6.25%, and 12.5%. In addition, 2.5% NaOCl was also used as a comparison group. The sample size group is based on calculations using the Federer formula, namely 5 samples, so that the total sample used are 25 samples.

Six kg of fresh green okra was washed and cut into small pieces and then dried without direct sunlight for 1 day, followed by drying in an oven for 3 days at 50°C. The dried green okra was mashed using a blender and then sieved and weighed. Using measuring cup, a total of 374.5 g of dark green okra powder was placed into a glass jar with a lid, and a 70% ethanol solution was added in a ratio (1:5 w/v) of 1872,5 mL. Maceration was carried out at room temperature for 72 hours. After that, the solution was filtered using filter paper. The filtrate obtained was concentrated using a rotary evaporator at a temperature of 50°C to obtain a thick extract of green okra (a concentration of 100%). Serial dilutions were carried out using sterile distilled water from the extract concentration of 100% to the desired concentrations of 1.563%, 3.125%, 6.25%, and 12.5%.

Brain heart infusion agar (BHI-A) media made by inputting 5.2 g of BHI-A powder and 100 mL sterile distilled water into an erlenmeyer tube and stirring until homogeneous then heating it on an electric stove until it boils; sterilize in an autoclave at 121ºC for 15 minutes. When the media is warm (40-50°C) poured into sterile petri dish with a thickness of 4 mm and let stand until solid. The BHI-A media was put into an anaerobic jar and incubated for 24 hours at 37°C.

Brain heart infusion broth (BHI-B) media made by inputting 3.7 g of BHI-B powder and 100 mL sterile distilled water into an erlenmeyer tube and then stirring with a spatula until homogeneous then heat it on an electric stove until it boils. Sterilize in an autoclave at 121ºC for 15 minutes and the media was incubated for 24 hours at 37°C.

The suspension of *E.faecalis* bacteria ATCC 29212 was made by taking 1 ose of bacterial culture and putting it into a test tube containing 2 mL of BHI-B. Put the test tube into an anaerobic jar and incubated for 24 hours at 37°C. Furthermore, standardization is carried out with a standard of 0.5 Mc.Farland ($1.5x10^8$ CFU/m).

The antibacterial test was carried out by the disc diffusion method. Each step of this treatment is carried out in laminar flow. Label each group on the bottom of the petri dish according to where the disc paper will be placed. Take 0.5 ml of the bacterial suspension from the test tube using a syringe, then drop it onto the BHI-A medium and spread it evenly using a streaking motion using a sterile cotton swab over the entire surface of the plate. The streaking movement was repeated three times and the petri dish was rotated 60º for each repetition. Wait for the media surface to dry for about 15 minutes. Soak each paper disc in a concentration of 1.563% dark green okra extract; 3.125%; 6.25%; 12.5% and 2.5% NaOCl until all parts are wetted (for 30 seconds). Place the paper disc using sterile tweezers on the surface of the media. The petri dish was closed and then put in an

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inverted position into an anaerobic jar, then incubated for 48 hours at 37°C.

Inhibition is known by measuring the diameter of the clear zone formed around the paper disc. Measurements were made using a digital caliper scale (mm).

RESULTS

Research on the inhibition of green okra extract against *E.faecalis* ATCC 29212 was carried out using the disc diffusion method. The amount of inhibition is known by measuring the diameter of the clear zone formed around the paper disc. The zone of inhibition can be shown in Fig 1 that shows from various concentrations of green okra extract, inhibition zones were not formed at concentrations of 1.563% and 3.125%, which was indicated by the absence of a clear zone around the paper disc. Inhibition zones began to form at concentrations of 6.25%, 12.5%, and 2.5% NaOCl.

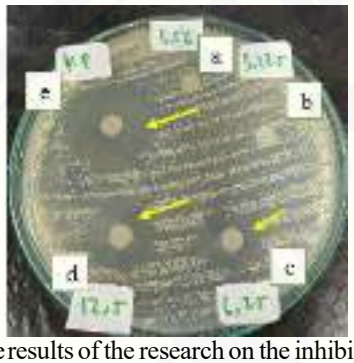

Figure 1 The results of the research on the inhibition of green okra extract against *E.faecalis* concentrations a) 1.563%, b) 3.125%, c) 6.25%, d) 12.5%, e) 2.5% NaOCl. The arrow indicates the inhibition zone formed.

The average diameter of the inhibition zone of each study group can be seen in Table 1 that shows the results of the calculation of the inhibition zone of green okra extract against *E.faecalis* ATCC 29212. The average the inhibition zone diameter starting from the smallest is 1.563% green okra extract (0 mm), 3.125% extract green okra (0 mm), 6.25% green okra extract (15.19 mm), 12.5% green okra extract (18.03 mm) and 2.5% NaOCl (24.07 mm).

Data analysis with the Shapiro Wilk normality test showed that the significance value obtained in the green okra extract concentrations of 6.25% and 12.5%, and 2.5% NaOCl were greater than 0.05 (p more than 0.05), meaning that the data is normally distributed. The results of the homogeneity test using the Levene test showed a significance value of 0.011 (p less than 0.05), meaning that the data were not homogeneous.

Furthermore, an analysis was carried out using a non-parametric Kruskal Wallis statistical test, that showed a significance value of 0.000 or the p-value less than 0.05. It means that there were differences between groups in the ability to inhibit *E.faecalis*. To determine the significance of the differences between groups, the Mann Whitney U test was performed that can be seen in Table 2; there were significant differences between all groups except between the green okra extract concentrations of 1.563% and 3.125%.

DISCUSSION

The results that green okra extract concentrations of 6.25 %, 12.5% and NaOCl 2.5% had inhibitory effects against *E.faecalis*. Concentrations of 1.563% and 3.125% had no inhibition as indicated by the absence of a clear zone around the paper disc. This was possible because there was small amount of active components so it was unable to inhibit *E.faecalis*.

According to Davis and Stout that cited by Mozartha et al, the inhibition of material is categorized into 4 categories, i.e. weak (≤ 5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>21 mm).[10] Based on Table 1, it is known that the inhibition of green okra extract concentrations of 6.25% (15.19 mm) and 12.5%

Table 1 The results of the measurement of the inhibition zone of ocra extract against *E.faecalis*

Research group	Inhibit zone diameter (mm)					Average
	Repetition					
	I	II	III	IV	V	
Extract 1.563%	0	0	0	0	0	0
Extract 3.125%	0	0	0	0	0	0
Extract 6.25%	14.40	15.20	15.40	15.60	15.35	15.19
Extract 12.5%	17.80	18.40	18.20	17.95	17.80	18.03
2.5% NaOCl	24.20	23.80	24.60	23.95	23.80	24.07

Table 2 Mann Whitney U test results

Research group	K1	K2	K3	K4	KP
K1 1.563% okra extract	-	1.000	0.005*	0.005*	0.005*
K2 3.125% okra extract	1.000	-	0.005*	0.005*	0.005*
K3 6.25% okra extract	0.005*	0.005*	-	0.009*	0.009*
K4 12.5% okra extract	0.005*	0.005*	0.009*	-	0.009*
KP 2.5 % NaOCl	0.005*	0.005*	0.009*	0.009*	-

(*) = Shows significant value

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(18.03 mm) belongs to the strong category while the inhibition of NaOCl is 2.5% (24.07 mm) belongs to the very strong category. The inhibition of green okra extract against *E.faecalis* increased along with the increase in extract concentration.[11]

Green okra extract can inhibit *E.faecalis* because it contains active compounds in the form of flavonoids, alkaloids, saponins, tannins, and terpenoids that have the potential as antibacterial. Flavonoids have several mechanisms, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism. Flavonoids inhibit nucleic acid synthesis by attacking DNA gyrase in bacteria that function in the process of DNA replication and transcription, to stop bacterial growth. The mechanism of flavonoids in inhibiting cell membrane function is by interfering with cell membrane permeability and inhibiting binding enzymes such as ATPase and phospholipase.[12]

Other compounds are saponins which have antibacterial effects because their surface-active substances are similar to detergents, so they can reduce the surface tension of cell walls and damage the permeability of bacterial membranes. The mechanism of action of alkaloids as an antibacterial is by disrupting the integrity of the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not completely formed and resulting in bacterial cell death. Tannin compounds have an antibacterial effect because they can deactivate bacterial cell adhesion, enzymes bound to cell membranes, and cell wall polypeptides so that bacterial cells are unable to carry out living or dead activities. The mechanism of action of terpenoids as an antibacterial is by break the bacterial cell membrane. And, terpenoids will bonding with transmembrane proteins (porins) on the outer membrane of the bacterial cell wall by forming a strong polymeric bond, which results in impaired permeability of the bacterial cell wall.[14,15]

NaOCl has a very strong inhibition against *E.faecalis*. In water, NaOCl ionizes to form sodium hydroxide (NaOH) and hypochlorous acid (HOCl). When in contact with organic tissue, NaOCl has three mechanisms of action i.e. saponification, neutralization, and chloramination. In the saponification reaction, NaOH will react with fatty acids (phospholipids) on the bacterial cell membrane which will break down fatty acids into fatty acid salts (soap) and glycerol, causing damage to bacterial cell membranes. When an amino acid is in contact with NaOH, a neutralization reaction will occur which will neutralize the amino acid into water and salt. Chloramination reaction occurs when hypochlorous acid contact with organic tissue (amino acids) and releases chlorine which is the active substance from NaOCl solution. Chlorine combines with amino acids to form chloramine. This reaction causes disturbances in the metabolism of bacterial cells by inhibiting bacterial enzymes, damaging DNA synthesis and hydrolyzing amino acids.[1,16]

Green okra extract concentration of 3.125% is not an effective concentration close to 2.5% NaOCl in inhibiting the growth of *E.faecalis*. Okra extract which is effective at close to 2.5% NaOCl is 12.5% concentration because it has the greatest inhibitory power among other concentrations. Green okra extract concentration of 3.125% was more effective in inhibiting gram-negative bacteria, namely *P.gingivalis* and *Aa* based on research by Luthfi et al. and Yuliati et al. while in this study the bacteria *E.faecalis* belongs to the gram-positive group.[8,9] The difference in antibacterial effectiveness against the two groups of bacteria can occur due to differences in the structure and components of the cell wall. The cell walls of gram-positive bacteria have a thick layer of peptidoglycan measuring 30-50 nm and contain teichoic acid. Gram-negative bacteria have a thinner peptidoglycan layer measuring 3-5 nm and do not contain teichoic acid so their cell walls are more susceptible to exposure to an antibacterial agent.[17,18]

The difference in the inhibition between green okra extract and 2.5% NaOCl could occur due to several things, i.e. the green okra extract in this study was still a whole extract, the active component that has antibacterial properties those cannot be separated purely from green okra. Whole extracts of green okra using ethanol as a solvent in the extraction process often contain undesirable compounds such as pigments (chlorophyll a and b, carotenoids, anthocyanins), carbohydrates, resins, and so on which disrupt the stability of the physical properties of the extract when it is formulated. Various kinds of component is suspected to be the cause of the reduced effectiveness as an antibacterial.[19] In contrast to 2.5% NaOCl which only consists of a few chemical elements such as sodium, oxide, and chlorine has a complex antibacterial mechanism through saponification, neutralization and chloramination reactions so that is more effective at inhibiting bacterial growth.[20]

Based on the research, it can be concluded that 6.25% and 12.5% green okra extract has inhibition against *E. faecalis*. 12.5% green okra extract is a concentration with an inhibitory strength close to 2.5% NaOCl, but there is no concentration of green extract that effectively inhibits *E.faecalis* equivalent to 2.5% NaOCl.

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