



Inhibitory power of green okra fruit extract (*Abelmoschus esculentus*) against *Streptococcus viridans*

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Abstract

Background: *Streptococcus viridans* (*S.viridans*) found in infected root canals with a prevalence of up to 63%. It is also found in 40-60% of cases of infective endocarditis, necessitating adequate cleaning during root canal treatment. NaOCl solution has been widely used as an irrigating agent because it has strong antibacterial properties, combined with EDTA which is able to soften inorganic tissue thereby increasing cleaning effectiveness. However, NaOCl can irritate soft tissue while EDTA has weak antibacterial ability. Green okra fruit contains antibacterial compounds that can inhibit the growth of bacteria. This research was conducted to compare the antibacterial power of green okra fruit extract with NaOCl and EDTA against *S. viridans*.

Method: Laboratory experimental research was carried out in vitro using a post test only control group design. Disc diffusion method was used with 7 sample groups (the extract variation group (100%, 50%, 25%, and 12.5%), 2.5% NaOCl, 17% EDTA, and distilled water negative control).

Result: The results of the average diameter obtained for each group are as follows green okra fruit extract concentrations of 12.5% (9.53 mm), 25% (12.64 mm), 50% (16.4 mm), and 100% (19.53 mm), 17% EDTA (21 .36 mm), 2.5% NaOCl (24.87 mm), and the distilled water (0 mm). The results of the Kruskal-Wallis statistical test showed ($p < 0.05$) which means there was a difference between groups. Man-Whitney test showed that there were significant differences between groups.

Conclusion: The results of the Kruskal-Wallis statistical test showed ($p < 0.05$) which means there was a difference between groups. Man-Whitney test showed that there were significant differences between groups.

Keywords: disc diffusion method; green okra fruit extract; inhibitory power; streptococcus viridans

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BACKGROUND

Streptococcus viridans (*S. viridans*) bacteria are most often found in infected root canals with a prevalence of 63% due to its virulence factors which include the ability to form biofilms, resistance to acids, its aciduric and acidophilic properties¹. *S. viridans* is found in 40%-60% of cases of infective endocarditis because this bacteria can enter the bloodstream via pulp infection². Therefore, root canal irrigation is important to create a microbial-free root canal thereby preventing the spread of infection³.

One of the irrigants that are widely used for root canal treatment is sodium hypochlorite (NaOCl) 2.5% and Ethylene diamine tetraacetic acid (EDTA) 17%. The use of these two materials is often combined in the root canal irrigation process because of their complementary way of working and increasing the effectiveness of cleaning³. 2.5% NaOCl solution is able to dissolve necrotic tissue and biofilm through saponification reactions, amino acid hydrolysis, and chloramination reactions, and has broad spectrum antimicrobial activity⁴. On the other hand, NaOCl can irritate

periapical tissues, swelling and irritation of the mucosa, even necrosis of soft tissues such as lips, labial and periapical mucosa⁵. Ethylene diamine tetraacetic acid (EDTA) works as a chelating agent to remove the smear layer of dentin, is able to form bonds with dentine calcium ions so that dentine becomes more easily destroyed and easier to instrument. EDTA works by binding Ca²⁺ and Mg²⁺ ions to the bacterial cell wall, thus damaging the function of the bacterial cell wall. However, EDTA has drawbacks, namely low antibacterial properties and EDTA extrusion causes disruption of the periapical inflammatory response^{6,7}.

Green okra fruit contains alkaloids, flavonoids, saponins, terpenoids and tannins which are useful as antioxidants, antidiabetic, antihyperlipidemic, antibacterial, anti-inflammatory and analgesic^{8,9}. Green okra fruit is proven to have higher antioxidant activity than red okra. Flavonoids can inhibit nucleic acid synthesis, cell membrane function, and metabolism in bacterial cells¹⁰. Alkaloids work by completely interfering with the mechanism of cell wall formation¹¹. Tannins and terpenoids work by interfering with the permeability of the bacterial cell membrane¹².

Yuliati et al., (2020) in their research proved that green okra fruit extract was able to inhibit *Porphyromonas Gingivalis* with a minimum inhibitory concentration (MIC) of 3.125% and a minimum bactericidal concentration (MBC) of 6.25%¹⁰. Research by Ermawati et al., (2023) showed that green okra fruit extract had an inhibitory effect on the growth of *S. viridans* bacteria with an extract concentration of 12.5% forming a smaller inhibition zone than 2.5% NaOCl¹⁴. This study aims to determine the comparison of the antibacterial activity of green okra fruit extract with 2.5% NaOCl and 17% EDTA on the growth of *S. viridans* bacteria.

RESEARCH METHOD

The type of research used was laboratory experimental in vitro using a post test-only control group design approach. The research sample was divided into 7 groups with varying concentrations of 100%, 50%, 25%, 12.5%, sterile distilled water negative control, 2.5% NaOCl group, 17% EDTA group. The treatment was repeated 5 times. The research was conducted from September to December 2022 at the Plant Laboratory, Department of Agricultural Production, Jember State Polytechnic, Surabaya Research and Industry Center, Laboratory Research Center, Dental and Oral Hospital, Airlangga University.

The materials used included green okra fruit extract (*Abelmoschus Esculentus*), 96% ethanol, sterile distilled water, 2.5% sodium hypochlorite (NaOCl) (*OneMed*), 17% EDTA (*Indodent*), *S. viridans* bacteria (Laboratory Research Center, RSGM) Unair), 0.5 Mcfarland standard solution, disc paper, cotton swab, filter paper, MHA powder (Mueller-Hinton Agar) (*Oxoid*), MHB powder (Mueller-Hinton Broth) (*Oxoid*). The tools used include petridishes, glass jar containers with lids, blenders (*Philips*), sieves, digital balances (*Tanita*), Erlenmeyer tubes (*Duran*), measuring cups (*Pyrex*), glass funnels, test tubes, Bunsen, reaction tube racks, paper labels, Spatula, Caliper, Rotary vacuum evaporator,

Incubator, Autoclave (*Hiramaya*), Laminar flow (*Sanyo*), Anaerobic jar (*Oxoid*), Tweezers, Electric stove (*IKA C-MAG HAS7*), Vortex, Buncher filter, Needle loops, Micropipet (*Socorex*).

Preparation of Green Okra Fruit Extract

Green okra pods are wet sorted and then washed, drained, cut into pieces, air dried and protected from direct sunlight at room temperature (25-30°C) for 12 days. After drying, the okra fruit is blended into a powder. The simplicia powder is then sifted and weighed. Using maceration method, The green okra fruit simplicia was extracted by 96% ethanol solvent with a ratio of 1: 2 for 3 x 24 hours with occasional stirring and remaceration was carried out 3 times. Maceration results are collected and filtered. The filtered results were mixed and thickened using a rotary vacuum evaporator at 40°C until a thick extract was obtained. Dilutions were made to obtain concentrations of 12.5%, 25%, 50%,

Media is prepared by mixing 3.7 g MHB powder with 100 mL of distilled water in an Erlenmeyer tube, stirred while heated until homogeneous, sterilized in an autoclave at 121°C for 15 minutes then the media is poured into a test tube. 3.8 g MHA powder mixed with 100 ml of distilled water in an Erlenmeyer tube, stirred while heated until homogeneous, sterilized in an autoclave at 121°C for 15 minutes¹⁵. then poured into a 4 mm thick petridish, put in an anaerobic jar and incubated at 37°C for 24 hours¹⁶.

One ose of *S. viridans* was taken from the stock and added to 4 ml of Mueller-Hinton Broth (MHB) medium. Incubate the MHB media that has been cultured with bacteria at 35°C for 24 hours and then adjust the turbidity until it reaches 0.5 McFarland or the equivalent of 1.5 x 10⁸ CFU/ml¹⁷.

The inhibition test was carried out using the disc diffusion method (Kirby-Bauer test). Drop paper discs with 10 microliter of 2.5% NaOCl solution, 17% EDTA, green okra fruit extract concentrations of 100%, 50%, 25%, and 12.5% using a micropipette and place it with a little pressure so that it sticks to the MHA media that have been inoculated. Place the petridishes in the incubator at 35°C 15 minutes after the discs have been placed on the agar medium for 24 hours. After incubation is complete, a clear zone will form around the disc paper. Measurement of the diameter of the inhibition zone using calipers with units of mm.

RESULTS

The results showed that an inhibition zone was formed around the disc paper containing green okra fruit extract concentrations of 12.5%, 25%, 50%, and 100% and the comparison group was 2.5% NaOCl and 17% EDTA. The results of the formation of the inhibition zone can be seen in Figure 1 below.

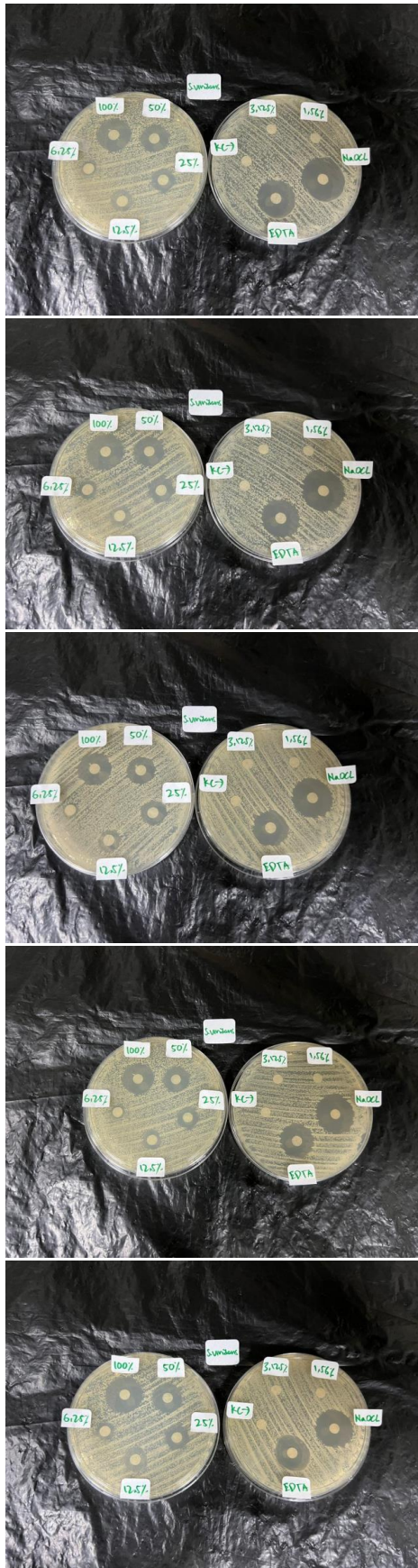


Figure 1. The results of the research on the inhibition of green okra fruit extract against *S. viridans* are indicated by red

arrows. 12.5%, 25%, 50%, 100%, 2.5% NaOCl, 17% EDTA, (K(-)) distilled water negative control.

The results of calculating the diameter of the inhibition zone of green okra fruit extract, the comparison group of 2.5% NaOCl and 17% EDTA against *S. viridans* can be seen more clearly in Table 1.

Table 1. Calculation results of the diameter of the inhibition zone

Sample Group	Mean ± SD
KP 1 (NaOCl 2.5%)	24,868 ± 0.53
KP 2 (EDTA 17%)	21.36 ± 0.28
K1 (12.5%)	9.53 ± 0.47
K2 (25%)	12.64 ± 0.58
K3 (50%)	16.4 ± 0.50
K4 (100%)	19.53 ± 0.72
K(-) (Aquadres)	0.0 ± 0.00

The largest diameter of the inhibition zone was formed around the disc paper which was dripped with 100% concentration of green okra fruit extract while the smallest inhibition zone was formed at a concentration of 12.5%. Paper discs dripped with sterile distilled water as a negative control did not show the formation of an inhibition zone. The average results for calculating the diameter of the inhibition zones of *S. viridans* in Table 1 are converted into a bar chart as shown in Figure 2.

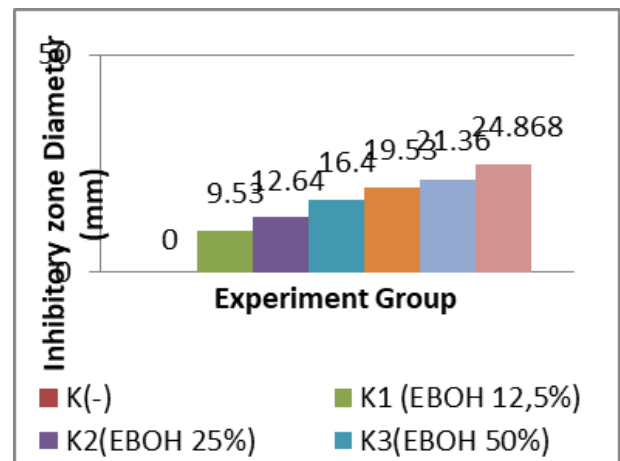


Figure 2. Bar chart of mean diameter of the inhibition zone of the green okra fruit extract group and the control group NaOCl 2.5% and EDTA 17%.

Based on the table and diagram above, it can be seen that the average diameter of the inhibition zone formed increased from concentrations of 12.5%, 25%, 50%, and 100%, EDTA 17% and the largest NaOCl 2.5%. The negative control group did not show any clear zones.

Based on the results of the Shapiro-Wilk normality test, a significance value of $\alpha > 0.05$ means that the data is normally

distributed. The homogeneity test using the Levene test shows a significance value of 0.000 ($p < 0.05$) meaning that the data is not homogeneous. The data were tested using the Kruskal-Wallis non-parametric statistical test because the data were normally distributed and not homogeneous. The results of this test showed a significance value 0.000 ($p < 0.05$), meaning that there were differences in inhibition in all groups.

The results of the Mann-Whitney statistical test showed that there was a significant difference between all groups ($p < 0.05$). This shows that the difference in concentration can affect the diameter of the inhibition zone formed.

Table 3. Mann-Whitney Test Results

Group	K1	K2	K3	K4	KP1	KP2	K(-)
K1		0.00 9*	0.00 9*	0.00 9*	0.00 9*	0.00 9*	0.00 5*
K2	0.0 09*		0.00 9*	0.00 9*	0.00 9*	0.00 9*	0.00 5*
K3	0.0 09*	0.00 9*		0.00 9*	0.00 9*	0.00 9*	0.00 5*
K4	0.0 09*	0.00 9*	0.00 9*		0.00 9*	0.00 9*	0.00 5*
KP 1	0.0 09*	0.00 9*	0.00 9*	0.00 9*		0.00 9*	0.00 5*
KP 2	0.0 09*	0.00 9*	0.00 9*	0.00 9*	0.00 9*		0.00 5*
K(-)	0.0 05*	0.00 5*	0.00 5*	0.00 5*	0.00 5*	0.00 5*	

*= there is a significant difference between groups

DISCUSSION

The results showed that green okra fruit extract concentrations of 12.5%, 25%, 50%, and 100% had an inhibitory effect on *S. viridans*. Based on the observed results, the diameter of the inhibition zone (shown in Table 4.1) that was formed showed an increase with increasing concentration of the extract. The smallest diameter of the inhibition zone was formed at an extract concentration of 12.5%, while the largest diameter was formed at an extract concentration of 100%. This can happen because the higher the concentration, the more bioactive components contained in the extract so that the ability to inhibit bacterial growth is also greater¹⁸.

The strength of the antibacterial activity can be determined based on the size of the inhibition zone formed using David and Stout's (1971) based on diameter, divided into 4 category ; highly strong >20 mm, strong 10-20 mm, moderate 5-10 mm, and weak <5 mm¹⁹. Based on the diameter of the inhibition zone shown in **Table 1**, the 12.5% concentration group is included in the moderate category, the 25%, 50%, and 100% concentration group is included in the strong category. The inhibition zone formed on 2.5% NaOCl and 17% EDTA is classified in the very strong category.

The antibacterial ability of green okra fruit is thought to be due to the active compounds of flavonoids, alkaloids, saponins, tannins, terpenoids, and steroids contained therein⁹. Green okra fruit contains 18% -22% of the total phenolic content of flavonoids, especially 70% quercetin²⁰. The flavonoid quercetin causes changes in the biophysical structure of the cell membrane which can prevent the formation of biofilms, and interferes with the cell wall biosynthetic mechanism, can cause damage to the cytoplasmic membrane, causing leakage and separation of the membrane and cell wall. Flavonoid quercetin damaging the plasma membrane by accumulating hydroxyl groups which change the hydrophobicity and surface charge of the membrane, triggering lipid breakdown, pore formation and leakage that leads into cellular disruption²¹. Biofilm formation by oral pathogenic bacteria such as *S. viridans* which is the main cause of caries and pulp infection can also be inhibited by quercetin. Biofilm formation is inhibited by interfering with the colonization surface and by decreasing cell attachment and extracellular protein secretion²².

Tannin compounds play a role in antibacterial activity by damaging bacterial cell membranes because they can form complex bonds with proteins that inactivate bacterial adhesin causing protoplasmic coagulation resulting in contraction of the bacterial cell wall, resulting in increased permeability of the bacterial cell membrane and cell wall, followed by intracellular leakage and ending lysis. Alkaloids as antibacterials work by interfering with the peptidoglycan arrangement of bacterial cells so that they interfere with the formation of a perfect cell wall¹¹. The antibacterial properties of saponins work by lowering the surface tension of the bacterial cell wall causing an increase in the permeability of the cell wall which results in cell leakage. Saponins diffuse through the cell membrane and cell wall then bind to the cytoplasmic membrane and disrupt the stability of the membrane, causing cell leakage which leads to cell death. Terpenoids contained in green okra fruit also play a role in antibacterial activity by breaking down lipophilic membranes thereby disrupting the integrity and permeability of cell membranes.²³

Based on the results of data analysis, it is evident that all concentration groups have a significant difference. EBOH and 2.5% NaOCl and 17% EDTA have different active ingredient content and mechanism of inhibition of bacterial growth which causes different antibacterial effects. The active ingredients contained in green okra fruit belong to the group of phenol compounds which are alcohol derivatives so that they evaporate easily and the resulting antibacterial effect is shorter, while NaOCl contains a very stable chlorine compound. Extracts using ethanol as an organic solvent often contain unwanted compounds such as chlorophyll pigments, carbohydrates, waxes, fibers, and various amino acids.²⁴. These various other ingredients can be the cause of reduced antibacterial activity. 2.5% NaOCl and 17% EDTA only consist of a few chemical elements that have strong antibacterial properties so that they can provide maximum antibacterial effects²⁵.

The 17% EDTA group and the 2.5% NaOCl group also showed a significant difference. The 17% EDTA inhibition zone is smaller than 2.5% NaOCl due to the specific antibacterial activity of EDTA on Gram-negative bacteria through the binding mechanism of Mg^{2+} and Ca^{2+} ions so that it can disrupt the stability of the cell wall and membrane of Gram-negative bacteria.⁷ *S. viridans* is a group of Gram-positive bacteria whose cell wall is composed of a thicker peptidoglycan layer, which is 30-50 nm, while the peptidoglycan layer in Gram-negative bacteria is thinner, namely 3-10 nm. Therefore, *S. viridans* bacteria are more resistant to 17% EDTA antibacterial activity¹⁶. The use of EDTA is used as a chelating agent which functions to dissolve the smear layer and other inorganic materials through the reaction of $Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \rightarrow 10Ca^{2+} + 6(HPO_4)^{2-} + 2H_2O$. EDTA will come in contact with the root canal walls (hydroxyapatite) and bind calcium ions, causing the release of calcium from the root canal walls. The dentine of the root canal will soften making it easier to prepare for obturation²⁶.

Irrigation solution made from 2.5% NaOCl is proven to have broad-spectrum antibacterial properties because it contains chlorine which functions to interfere with bacterial metabolism. When NaOCl comes into contact with water, the reaction will occur $NaOCl + H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^+ + OH^- + H^+ + OCl^-$. This reaction forms hypochlorous acid which causes a reduction-oxidation reaction to produce hydroxyl radicals, hydrogen peroxide and superoxide. The compounds formed are capable of denaturing proteins, oxidizing membrane lipids and bacterial cell walls, causing enzyme deactivation, and damaging bacterial DNA. Release of active chlorine can oxidize -SH groups in essential bacterial enzymes thereby disrupting bacterial metabolic functions²⁷.

The weakness of this study is that the results obtained are still qualitative results by categorizing the antibacterial properties of green okra fruit extract into three categories, namely, very strong, strong, and moderate. The clear zone that forms around the disc can be interpreted as an obstacle to bacterial growth, but cannot be interpreted as bacterial death. In addition, the results of this study cannot be used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts because it is very difficult to measure the amount of extract that diffuses through the agar medium. The approximate MIC can be calculated by comparing the diameter of the inhibition zone formed with certain standard algorithms²⁸. Therefore, further research is needed to determine the MIC and MBC of green okra fruit extract and their antibacterial effects on *S. viridans*.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that green okra fruit extract (*Abelmoschus esculentus*) concentrations of 12.5%, 25%, 50%, and 100% have less inhibition on the growth of *S. viridans* (significantly different) than NaOCl 2.5% and EDTA 17%.

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