ORIGINAL ARTICLE

Analyzing the antibacterial ability of green okra fruit (*Abelmoschus esculentus* L. Moench.) extract at several concentrations against *Staphylococcus aureus*: an experiment study

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ABSTRACT

Introduction: The common bacteria found in infected root canals are Gram-positive bacteria such as *Staphylococcus aureus* (20%). The bacterial infection can spread to the periapical tissues causing periapical lesions. Pulp necrosis, the cause of periapical lesions, requires root canal treatment. *Ethylenediaminetetraacetic* acid (17% EDTA) is one of the irrigants that can be used for root canal treatment however, 17% EDTA has a low antibacterial effect. The green okra fruit extract contains antibacterial properties that can inhibit the growth of bacteria. This study aims to analyze the antibacterial ability of green okra fruit extract at concentrations of 12.5, 25, 50, and 100% against *S. aureus* using a negative and positive control. **Methods:** This type of research was an *in-vitro laboratory* experiment with a posttest-only control group design. The antibacterial test using the disc diffusion method consisted of six groups: green okra fruit extract concentrations of 12.5, 25, 50, 100, and 17% EDTA (positive control), and aquadest (negative control). Antibacterial ability is indicated by the clear zone produced around the disc paper. **Results:** The average diameters of the inhibition zone were the green okra fruit extract concentrations of 12.5% (12.14), 25% (14.89), 50% (18.53), 100 (21.1), and 17% EDTA (22.08 mm), and 0 mm for aquadest. The results were analyzed using the Mann-Whitney test and showed a significant difference between all research groups ($p<0.05$). **Conclusion:** The antibacterial ability of green okra fruit extract *(Abelmoschus esculentus* L. Moench) against S. aureus increased with increasing concentrations, but remained lower than the antibacterial ability of 17% EDTA.

KEYWORDS
antibacterial activity, green okra, *staphylococcus aureus*

INTRODUCTION

Caries in permanent teeth that are left untreated may lead to dental pulp infections, such as pulp necrosis. One type of bacteria found in the root canals of necrotic teeth is *Staphylococcus aureus* (20%).[1] Bacterial infection in pulp necrosis can spread to periapical tissues, causing periapical lesions, such as acute apical periodontitis, periapical abscess, and chronic apical periodontitis.[2] The percentage of *S. aureus* bacteria in periapical abscesses may reach 35.63%.[3] The infection can spread more widely to cause severe diseases, such as Ludwig's angina.[4] Therefore, the elimination of *S. aureus* in the root canal is necessary to prevent further disease.

Pulp necrosis requires root canal treatment (RCT).[5] There are three main stages in RCT: biomechanical preparation, sterilization, and root canal filling. Irrigation is required at the biomechanical preparation and sterilization stages to clean the dentin that remains after preparation, dissolve necrotic tissue, and provide lubricant.[6] The most common irrigants is *ethylenediaminetetraacetic acid* 17% (17% EDTA).[7] The EDTA solution is used during root canal preparation and is used interchangeably with sodium hypochlorite (NaOCl).[8] Ideally, irrigants have antibacterial properties, the ability to dissolve necrotic tissue, and have a low level of toxicity.[9]

Seventeen percent of EDTA solution can soften root canal dentin because it can dissolve the inorganic components of the smear layer.[10] In contrast to NaOCL, which has a broad spectrum of the antibacterial activities, 17% EDTA has lower antibacterial power. For optimal results, EDTA is usually applied for one minute, followed by NaOCl. On the other hand, dentin that is exposed to high concentrations of EDTA for an extended period of time undergoes extensive demineralization in the root canal.[6] In addition to its disadvantages, EDTA is relatively expensive.

Several chemical agents in EDTA cause changes in the microchemistry and mechanical properties of dentin, potentially altering the original proportions of organic and inorganic components

by modifying the microhardness.[11] The shortage of synthetic irrigants has led to the need for alternative irrigation materials.

One of the natural ingredients that can be used as alternative irrigation is green okra fruit *(Abelmoschus esculentus).* Green okra fruit extract contains antibacterial compounds such as alkaloids, flavonoids, saponins, tannins, and terpenoids.[12,13] Green okra fruit has been reported to possess several biological activities, such as anti-inflammatory, antibacterial, antipyretic, antidiabetic, and anticancer.[14]

Research on the antibacterial ability of okra fruit extract is one of them conducted by Vitasari et al[15], regarding the inhibition of okra fruit extracts concentrations of 1.56, 3.125, 6.25, and 12.5% compared to the inhibition of 2.5% NaOCl against the growth of Staphylococcus aureus. Vitasari et al[15]., conducted a comparison study on the inhibitory effects against S aureus growth through the antibacterial ability of okra fruit extracts at concentrations of 1.56, 3.125, 6.25, 12.5, and 2.5% NaOCL. The result showed an okra fruit extract concentration of 12.5% (17.95 mm) had a diameter of inhibition zone close to 2.5% NaOCl (24.56 mm). This indicated the lack of synthetic root canal irrigants and the presence of secondary metabolites in okra fruit extract that are antibacterial. This study aims to analyze the antibacterial ability of green okra fruit extract at concentrations of 12.5, 25, 50, and 100% against S. aureus using a negative and positive control.

METHODS

This research was an *in-vitro laboratory* experiment with a posttest-only control group design. The Green okra fruit samples were obtained from PT. Mitratani Dua Tujuh Jember, then identified at the Plant Laboratory, Jember State Polytechnic. Green okra fruit was extracted using the maceration method with 96% ethanol. Green okra fruit samples were washed and cut into pieces, then dried at room temperature without direct sunlight. The dried samples were blended into a powder and sieved using a 40-mesh sieve. 627 grams of green okra fruit simplicia were put into a jar, and 96% ethanol (1245mL) (1:2 w/v) was added. The maceration process was carried out for 3x24 hours at room temperature, and then the filtrate was concentrated at 40^0C using a rotary vacuum evaporator to obtain a 100% thick extract.

In this study, the green okra fruit extract was diluted using the serial dilution method by adding aquadest. S Using distilled water, the concentration of the extract was diluted from 100 percent to the desired concentrations of 12.5, 25, and 50%. The research sample consisted of six groups with the following concentrations of green okra fruit extract: 12.5, 25, 50, 100, EDTA 17% (positive control) and aquadest (negative control). Each sample was repeated five times.

The bacterial suspension was made by taking an oese of *S. aureus* from the culture media and then inoculating it in a test tube containing 2 ml of Mueller Hinton Broth (MHB). The test tube was incubated at 37^0C for 24 hours. After incubation, the bacterial suspension was standardized according to the 0.5 McFarland standard (1.5 x 10^8 CFU/ml).

The antibacterial test used the disc diffusion method. The suspension of *S. aureus* was evenly inoculated on Mueller Hinton Agar (MHA) media using a sterile cotton swab. The paper discs were dropped with ten microliters of green okra fruit extract concentrations of 12.5, 25, 50, 100 percent, 17% EDTA, and aquadest. Using tweezers, the disc paper was slightly pressed onto the MHA media. After that, it was incubated for 24 hours at 37^0C. The antibacterial activity could be observed in the large diameter of the disc paper's clear zone. The diameter of the clear zones was measured using calipers. Measurements were taken two times (vertically and horizontally) before dividing the results in half to get the average.

The data obtained were analyzed using the SPSS v.25. The normality test was determined using the Shapiro-Wilk test. The homogeneity of the variance was evaluated using the Levene test. Furthermore, an analysis was carried out using a non-parametric Kruskal-Wallis test and the Mann-Whitney test.

RESULTS

The antibacterial ability of green okra fruit extract against *S. aureus* through the diameter of the inhibition zone was seen as a clear zone around the disc paper (Figure 1).

Figure 1. The antibacterial ability of green okra fruit extract against *S. aureus* is shown as a clear zone around the paper disc (red arrow). A) Inhibition zone of green okra fruit extract at a concentration of 12.5%, B) Inhibition zone of green okra fruit extract at a concentration of 25%, C) Inhibition zone of green okra fruit extract at a concentration of 50%, D) Inhibition zone of green okra fruit extract at a concentration of 100%, E) Inhibition zone of positive control (EDTA 17%), F) negative control (aquadest), X) Concentrations are not used as samples.

Table 1. The average diameter of the inhibition zone and standard deviation

Research groups	Repetitions (mm)					Average ± SD
	I	II	III	IV	V	
K(-) (aquadest)	0	0	0	0	0	0.001±0.001
Extract 12.5%	12.4	12.35	11.95	11.8	12.2	12.14±0.25
Extract 25%	15.05	16.2	15.2	13.4	14.6	14.89±1.01
Extract 50%	18.2	19.05	18.8	18.4	18.2	18.53±0.38
Extract 100%	21.4	20.95	21.6	20.6	20.95	21.1±0.39
K(+) (EDTA 17%)	22.2	21.8	22.2	21.8	22.4	22.08±0.26

The inhibition zone diameters of green okra fruit extract increased with increasing concentrations. The average diameters of the inhibition zone from the smallest to the largest were the green okra fruit extract concentrations of 12.5% (12.14 mm), 25% (14.89 mm), 50% (18.53 mm), 100% (21.1 mm), and the positive control group (22.08 mm) (Table 1). The negative control did not form an inhibition zone (0 mm).

The results were tested for normality using the Shapiro-Wilk test. The results obtained a significance value of $p>0.05$, so the data is normally distributed. Then, a variant homogeneity test was carried out using the Levene test to test the population variance. Levene test results obtained a significance value of 0.017 ($p<0.05$), so the data is not homogeneous. Furthermore, the Kruskal Wallis nonparametric statistical test was carried out to determine whether there were differences in antibacterial ability in all research groups. The Kruskal-Wallis test results obtained a significance value of 0.001 ($p<0.05$), meaning there were differences in antibacterial ability in all research groups. The test was then continued with the Mann-Whitney test to find whether a significant difference between all research groups could be observed. The results showed that there was a significant difference ($p <0.05$) between all study groups (Table 2).

Table 2. Mann-Whitney test results

Research groups	K(-) (aquadest)	Extract 12.5%	Extract 25%	Extract 50%	Extract 100%	K(+) EDTA 17%
K(-) (Aquadest)		0.005*	0.005*	0.005*	0.005*	0.005*
Extract 12.5%			0.009*	0.009*	0.009*	0.009*
Extract 25%				0.009*	0.009*	0.009*
Extract 50%					0.009*	0.008*
K(+)(EDTA 17%)						

* = There were significant differences between all research groups

DISCUSSION

The results showed that the green okra fruit extract concentrations of 12.5, 25, 50 and 100% had antibacterial ability against *S. aureus*. The diameters formed at concentrations of 12.5% (12.14 mm), 25% (14.89 mm), and 50% (18.53 mm) were classified as strong, while at 100% (21.1 mm) concentration was categorized as very strong.[16] The results of this study were in line with the research of Martina *et al.*[13], who claimed that the antibacterial ability produced was different at each concentration. The higher the dilution, the lower concentration of secondary metabolites contained therein, so the diameter of the inhibition zone formed was smaller. These secondary metabolites are alkaloids, flavonoids, saponins, tannins, and terpenoids.[12]

Alkaloids can interfere with the constituent components of peptidoglycan in bacterial wall cells. If disturbed, it will cause the cell wall layer not to form completely and cause cell death. Terpenoids cause damage to the bacterial cell wall. If it is damaged, it can reduce the permeability of the bacterial cell wall, and the bacteria will lack nutrients, then their growth will be stunted or die.[17] Flavonoids contain phenolic compounds that are acidic, so they can interfere with bacterial growth by denaturing proteins and damaging the bacterial cell membrane. Metabolic activity will stop, which can result in bacterial cell death. Saponins as antibacterials can increase the permeability of cell membranes resulting in hemolysis. Tannins can interfere with the formation of the bacterial cell wall so that it becomes imperfect and results in the death of the bacterial cell.

The smallest inhibition zone of the green okra fruit extract group was 12.5% resulting in a 12.14 mm diameter zone. Meanwhile, the largest and strongest inhibition zone of the green okra fruit extract group was 100%, with a 21.1 mm diameter zone. The difference in the inhibition zone diameters at those concentrations was due to the differences in the secondary metabolites contained therein. The negative control group (aquadest) did not form inhibition zones. It means that aquadest does not have antibacterial ability. Meanwhile, the positive control group's (17% EDTA) average inhibition zone diameter was 22.08 mm. 17% EDTA solution can inhibit the growth of S.aureus because of its ability to damage bacterial cell membranes.[18] As a chelating agent, 17% EDTA solution can chelate Ca^{2+} and Mg^{2+} cations, thus decreasing their availability in cell membranes. Ca^{2+} and Mg^{2+} cations play a role in connecting lipopolysaccharide (LPS) to the cell wall of Gram-negative bacteria, whereas in Gram-positive bacteria, they play a role in connecting teichoic acid as a cell constituent.

The reduction in these cations can disrupt the stability of the bacterial cell membrane and cause the release of cell membrane components.[19]

The antibacterial ability of 17% EDTA was greater than green okra fruit extract (Table 1). The results of the Kruskal-Wallis test also showed a significant difference ($p<0.05$) between the antibacterial activity of green okra fruit extract and the antibacterial activity of 17% EDTA. This may be due to the fact that the material has, through various processes, acquired antibacterial properties, which are essential for irrigating agents. EDTA solution is a polyamino carboxylic acid $[CH_2N(CH_2CO_2H)_2]_2$ that can bind metal ions through four carboxylic groups and two amine groups. The ability of EDTA to chelate metal ions influences its antibacterial properties. Meanwhile, the green okra fruit extract in this study was still at an early stage and had not been purified or fractionated, so the resulting extract was a crude extract. The crude extract of green okra fruit still contains unnecessary compounds, such as pigments (chlorophyll a and chlorophyll b, carotenoids, anthocyanins), carbohydrates, and wax. These various compounds can cause the extract's physical instability and may reduce its antibacterial ability.[20]

The research on the antibacterial ability of green okra fruit was also performed on other bacteria found in the root canals of necrotic teeth, such as *Enterococcus faecalis*.[20] Elimination of the bacteria in the root canal affects the success of root canal treatment and prevents bacterial invasion of the periapical tissues.[21] Green okra fruit extract has demonstrated the ability to inhibit the growth of these bacteria. Therefore, green okra fruit extract can be considered and developed as an alternative to root canal irrigation or root canal medicament. This consideration is based on green okra fruit extract, which has been reported in several biological activities, such as anti-inflammatory, antibacterial, antipyretic, antidiabetic, and anticancer.[14] Other research states that okra fruit extract has significant activity in wound healing.[12]

CONCLUSION

The antibacterial ability of green okra fruit extract *(Abelmoschus esculentus* L. Moench*)* against *S. aureus* increased with increasing concentrations, but remained lower than the antibacterial ability of 17% EDTA

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