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Phytochemical Analysis and Antibacterial Property of Green Okra Fruit Extract Against Enterococcus Faecalis Bacteria Tooth Root Canals

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ABSTRACT

Background: Root canal treatment aims to eliminate bacteria that cause pulp abnormalities. Enterococcus faecalis is a bacteria in the infected tooth pulp, even those that have been treated. Irrigation was carried out together with biomechanical preparation using a combination of NaOCl and EDTA. The material is antibacterial, dissolves the organic and inorganic tissue of the root canal dentin, lacks odor and irritates the soft tissue. An alternative irrigation material from Jember cultivation is green okra. The fruit contains flavonoids, tannins, steroids, terpenoids. This study aims to analyze the phytochemical and antimicrobial properties of green okra fruit extract on the growth of E. Faecalis bacteria.

Method: Phytochemical analysis to see the content of alkaloids, flavonoids, tannins, saponins, steroids and terpenoids. Antimicrobial analysis included the inhibition test using disc diffusion, MIC and MBC tests using the dilution method. Samples were green okra fruit extract 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.563%, 2.5% NaOCl, 17% EDTA. The MIC and MBC tests were examined for Optical Density values with a spectrophotometer and then subcultured to see whether there was growth of bacterial colonies. Analysis of the research data used ANOVA and LSD parametric statistical tests

Result: Phytochemical content sequentially is Polyphenols, Alkaloids, Flavonoids, Saponins, Tannins, Steroids, Terpenoids. Antimicrobial properties green okra fruit extract concentration of 6.25% had the smallest inhibition zone, the largest OD value and the highest colony count. The concentration of 100% has the largest inhibition zone, the lowest OD, there are no bacterial colonies. Antimicrobial power The concentration of 100% is close to that of 17% EDTA and 2.5% NaOCl. In the *Kruskal Wallis* test, there were differences in the *Mann-Whitney* test study group, there were significant differences between all groups

Conclusion: The phytochemical content of green okra fruit extract has antimicrobial activity against *E. faecalis* approaching 2.5% NaOCl and 17% EDTA at 100% concentration, 6.25% MIC concentration and 12.5% MBC concentration.

Keywords: *Phytochemical, Antibacterial, Green okra fruit extract, Enterococcus faecalis*

INTRODUCTION

In Indonesia, pulp disease was the seventh most common disease among outpatient hospital visitors in 2010 with 163,211 visits.[1] Pulp disease begins with bacterial

invasion from the carious lesion into the pulp tissue, causing microbial dysbiosis in the pulp and root canal. Dysbiosis triggers inflammation and necrosis. The main bacteria that trigger this dysbiosis are believed to be facultative anaerobic bacteria, such as *Streptococcus viridans* and *Enterococcus* faecalis.[2,3]

Enterococcus faecalis is a bacterium that is responsible for about 85%-90% of infections in pulp tissue and root canals. *Enterococcus faecalis* can ferment carbohydrates into lactate, malate, citrate, arginine, agmati, and acid. In an in vitro study, *Enterococcus faecalis* can invade the dentinal tubules, which not all bacteria have this ability. *Enterococcus faecalis* is also a bacterium that can enter the survival phase but does not multiply.[4]

Pulp disease is a major challenge because not only the progressiveness of the disease can reduce the quality of life, but people are more likely to avoid treatment, due to repeated visits and expensive treatment costs. This is the basis for research on alternative therapies and materials for dental caries treatment, especially materials that are antibacterial, immunomodulatory and regenerative agents, to prevent the development of caries and repair damaged dental hard tissues[5,6].

The treatment that can be done on teeth with pulp necrosis is root canal treatment. Root canal treatment is a treatment that aims to thoroughly clean the root canal from infected pulp tissue so that the root canal chamber can be shaped and prepped for filling. Root canal treatment includes three stages, namely biomechanical preparation, sterilization and root canal filling. The preparation and sterilization stage requires root canal irrigation material which functions to remove necrotic tissue, debris, and kill microorganisms in the root canal.

Chemicals that are commonly used today as irrigation agents include NaOCl, EDTA and Chlorhexidine. They are root canal irrigation agents with a broad antimicrobial spectrum that can eliminate both Gram-positive and Gram-negative organisms. The mechanism of action in eliminating bacteria is by interacting with phospholipids and lipopolysaccharides on the bacterial cell membrane. These materials can effectively eliminate bacteria, but are toxic and odorous.[4] One of the root canal irrigation materials currently used is NaOCl 2.5% due to its ability to dissolve necrotic pulp tissue, rinse out debris from the root canal and is broad-spectrum antimicrobial. However, the use of 2.5% NaOCl also has its drawbacks, namely an unpleasant odor and taste, causing irritation when it gets pushed into the periapical tissue, and unable to dissolve inorganic components from the smear layer.[7] The use of EDTA as a root canal irrigation material is done to complement the mechanism of action of NaOCl. EDTA has the ability to dissolve root canal dentin tissue but its antibacterial properties are lower than NaOCl. EDTA forms a calcium-chelate solution with dentin calcium ions so that dentin becomes more fragile and easier to be instrumented. It is widely used during the root canal cleaning and shaping process as it is effective in achieving canal patency, canal dilation and smear layer removal.

Green okra (*Abelmoschus esculentus*) is a vegetable originating from Hindi bhindi. The health benefits of okra include preventing diabetes, lowering cholesterol, preventing the development of cancer, and being good for the digestive system. The unripe, fresh green fruit can be eaten immediately, but is usually consumed as a cooked vegetable. The fruit develops very quickly and can be harvested a week after the flowers appeared. Studies have shown that okra contains important bioactive compounds such as carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid, and amino acid.[8] Okra has low saturated fat and provides sufficient amounts of minerals. Okra contains flavonoids, saponins, steroids, alkaloids and tannins that are useful in many CNS disorders. Okra extract contains substances that affect intracellular Ca2+ metabolism ion elevation in certain doses, the release of proinflammatory mediators. However, the

content of green okra fruit in Jember has not been explored. Natural conditions, soil, climate and treatment during cultivation greatly affect the active components contained in the plant.[9,10]

Therefore, this study aims to analyze the phytochemical and antibacterial properties of green okra fruit (*Abelmoschus esculentus*) against *E. faecalis* bacteria in dental root canals. It is hoped that if the antibacterial ability is good, it will be used as an alternative root canal medication in the years to come.

MATERIALS & METHODS

This research is an experimental laboratory with post control group design. The independent variable is green okra fruit extract. The dependent variables were phytochemical properties and antibacterial activity. All research procedures were approved by the ethics commission of the Faculty of Dentistry, University of Jember number 1766/UN.25.8/KPEK/DL/2022.

Preparation of Green Okra Fruit Extract was done by cutting the okra fruit into small pieces, drying it (aerated for 2 days in room temperature), then oven for 24 hours at 400°C. The dried results were then blended and sieved with an 80 maze sieve until they became fine powder. The powder was then macerated with 96% ethanol for 3 days and stirred every 24 hours. Then the solution was concentrated with a rotary evaporator with a temperature of 500°C and a rotation of 90 RPM until a paste-like extract was obtained.

Phytochemical Analysis of Green Okra Fruit Extract was done to determine the content of alkaloids, flavonoids, tannins, saponins, steroids and terpenoids. Alkaloid test is done by mixing 0.5 g of extract with distilled water in a test tube, heated for 2 minutes, cooled and filtered, so that a filtrate is formed. Then the filtrate was given 2-3 drops of Mayer's reagent. Positive results are indicated by the formation of a white precipitate.

For flavonoid test, the filtrate is mixed with magnesium powder (Mg), 2N hydrochloric acid and amyl alcohol, and shaken firmly. When the color changes to red, yellow, and orange are indicators of the presence of flavonoid compounds. Saponin test, 0.5 g of green okra fruit extract is done by placing it in a test tube, adding 10 mL of hot distilled water, shaken vigorously for 10 seconds, and the presence of saponins is indicated by the formation of foam that does not disappear after 10 minutes.

The phenol test of 0.5 g of green okra fruit extract was done by inserting it into a test tube, adding 1-2 drops of 1% iron (III) chloride reagent, if the color changed into green, blue or blackish, it indicated the phenol content. Tannin test, done by adding distilled water to the extract, followed by 2-3 drops of 10% NaCl and 2-3 drops of FeCl. Blue green color changes (catechol tannins) and black blue (pyrogalol tannins) indicate positive results for tannin content. Steroid and terpenoid test, 0.5 g was done by dissolving green okra fruit extract in 0.5 ml chloroform, then adding 0.5 ml acetic anhydride and 2 ml concentrated sulfuric acid. Blue or green color changes indicate steroid compounds, and red, pink or purple indicate the presence of terpenoids.

Inhibition test was conducted using disc diffusion method. Bacteria were inoculated on a petridish containing solid MHA media, then discs that had been dripped with 10 µl of green okra fruit extract 100%, 50%, 25%, 12.5%, 6.25%, 3.175%, 1.5625%, NaOCL 6.25% and EDTA 17% were affixed to the surface of the media with tweezers. Petridish was incubated at 37°C for 24 hours. Next, the inhibition zone was measured with a caliper. Measurements were made 3 times on different sides. The inhibition test results will be classified into 3 categories: 11-15 mm diameter = resistant; 16-20 mm = intermediate; and > 20 mm = sensitive.[11]

Minimal Inhibition Concentration and Minimal Bactericidal Concentration tests were conducted by dilution method. *E. Faecalis* bacteria were inoculated on 10 mL of MHB media in Erlenmeyer, then incubated for 24 hours and then shaken at

150 RPM. After 24 hours, 200 µL of inoculum was put into a test tube containing 5 mL of MHB media. then 100 µL of green okra fruit extract 100%, 50%, 25%, 12.5%, 6.25%, 3.175%, 1.5625%, NaOCL 6.25% and EDTA 17% were put into a test tube containing 105 CFU (colony forming unit) bacterial inoculum. Then the test tube was incubated for 24 hours at 37°C. E. faecalis in the tube was subcultured. If in the follow-up test there is growth of bacterial colonies, then the concentration is declared as the MIC value. Meanwhile, if there is no growth of bacterial colonies in the follow-up test, the concentration is declared as the MBC value.

STATISTICAL ANALYSIS

Analysis of the research data used ANOVA and LSD parametric statistical tests.

RESULT

Phytochemical test results of green okra fruit extract in order from large to small percentage are Polyphenols 8.11%, Alkaloids 6.88%, Flavonoids 5.01%, saponins 4.02%, tannins 3.81%, steroids 3.22%, terpenoids 2.95%. Antibacterial is measured by measuring the diameter of the inhibition zone in the form of a clear zone formed around the disc paper and the number of bacterial colony growth

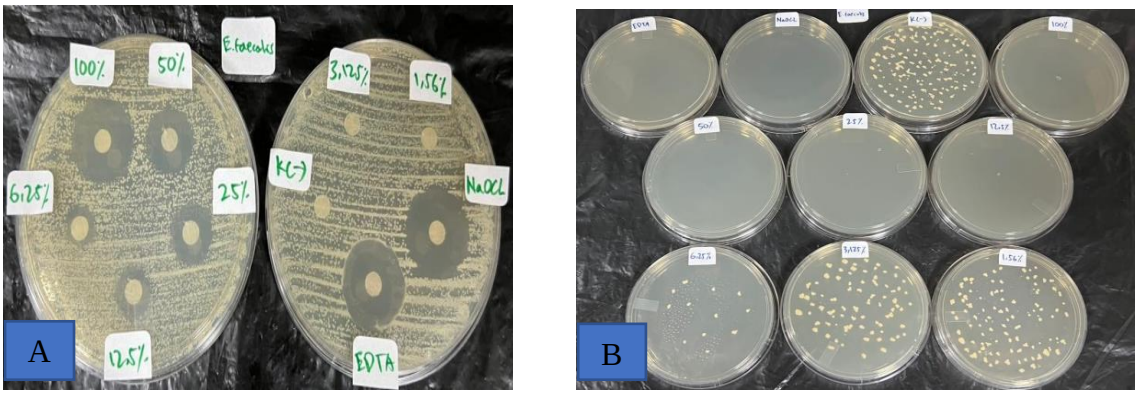

Figure 1 Antibacterial result of okra fruit extract against *E. faecalis*. A. Zone of inhibition; B. Colony count

The average diameter of the inhibition zone, optical density value and colony count of green okra fruit extract against E. faecalis can be seen in table 1.

Table 1. Average Zone of Inhibition Diameter, Optical Density and Colony Counts

No	Types of diseases	Zone Diameter	Optical Density	Colony Counts
1.	EDTA 17%	20.7500	.0850	.0000
2.	NaOCl 2,5%	24.2500	.0526	.0000
3.	Aquades K (-)	.0000	.9700	155.6000
4.	Green okra fruit extract 100%	19.2800	.1394	.0000
5.	Green okra fruit extract 50%	16.6400	.2698	.0000
6.	Green okra fruit extract 25%	14.3900	3756	.0000
7.	Green okra fruit extract 12,5%	9.6300	.5280	.0000
8.	Green okra fruit extract 6,25%	8.1500	.7168	12.4000
9.	Green okra fruit extract 3,125%	.0000	.8230	52.4000
10.	Green okra fruit extract 1,56%	.0000	.9468	117.0000

The results of the Shapiro-Wilk normality test and homogeneity Levene Test showed that the data were normally distributed and not homogeneous. Furthermore, the data was tested nonparametrically with Kruskal Wallis to determine the difference between groups, the significance value was 0.000 ($p<0.05$) meaning that there was a difference between groups. Then, Mann-Whitney test was conducted to find out which group had a significant difference. The results of the Mann-Whitney test showed a significance value of 0.000 ($p <0.05$), meaning that there were differences

between all groups in terms of inhibition zone diameter, optical density value and colony count.

DISCUSSION

Optical Density (OD) value is a value that indicates the high and low growth or population of bacteria in a medium. The growth of microorganisms can be measured by looking at cell concentration (number of cells per unit culture content or CFU) and cell density (dry weight of microorganism cells per unit culture content). Optical density counting is measured using a spectrophotometer. The number of colonies was counted using a colony counter. To determine the relationship between the level of turbidity and the number of bacterial colonies that grow, the Optical density (OD) test was conducted to calculate the absorbance value.

The positive control NaOCl 2.5% had the highest inhibition zone diameter (24.25 mm), OD value (0.0526), and there was no colony growth of E. faecalis. This means that the material has the ability to inhibit E. faecalis categorically sensitive, low bacterial growth in the media (low bacterial density), indicated by subculture there is no growth of bacterial colonies. This is because NaOCL ionizes into sodium hydroxide (NaOH) and hypochlorous acid (HOCl) after mixed with water. The mechanism of action of NaOCl when in contact with organic tissue is saponification, neutralization, and chloramination. Saponification reaction, NaOH will react with phospholipids of bacterial cell membranes, then break down phospholipids into fatty acid salts (soap) and glycerol. This causes damage to the bacterial cell membrane. When amino acids are in contact with NaOH, a neutralization reaction will occur, which will neutralize the amino acids into water and salts. The chloramination reaction occurs when hypochlorous acid contacts organic tissue (amino acids) and releases chlorine which is the active substance of the NaOCl solution. Chlorine that combines with amino acids will form chloramines. This reaction causes interference with bacterial cell metabolism by inhibiting bacterial enzymes, damaging DNA synthesis and hydrolyzing amino acids.[7,12]

Green okra fruit extract 100% has an inhibition zone diameter (19.28mm), OD value (0.1394), and there is no colony growth of E. faecalis. This means that the material has the ability to inhibit E. faecalis in the intermediate category, the growth of bacteria in the media is low (low bacterial density), indicated by the subculture with no bacterial colony growth. The antibacterial ability of 100% green okra fruit extract is still below NaOcl 2.5% and EDTA 17%. This is because 100% green okra fruit extract according to the results of phytochemical tests has the highest concentration of bioactive substances, mainly phenol compounds (8.11%), the hydroxyl group of phenol compounds (OH) affects the antibacterial activity in inhibiting bacteria. Phenol compound without hydroxyl groups has higher antibacterial activity because it can increase its ability to bind with lipid membranes. The level and number of hydroxyl (OH) functional groups in the phenol group are related to the level of toxicity to microorganisms, the more the hydroxylation process increases, the level of toxicity also increases. The higher the oxidized phenol compound, the stronger the inhibition of microorganism growth. The mechanism of phenol toxicity to microorganisms is through the process of enzyme inhibition by oxidized compounds, reactions with sulfihydryl groups or non-specific interactions with proteins. In addition, phenol compounds can cause protein denaturation through an adsorption process involving hydrogen bonds.[13]

Other compounds contained in 100% green okra fruit extract are Alkaloids 6.88%, Flavonoids 5.01%, Saponins 4.02%, Tannins 3.81%, Steroids 3.22%, Terpenoids 2.95%. These compounds have different mechanisms in inhibiting bacterial growth. Alkaloids interfere with the constituent components of peptidoglycan in bacterial

cells so that the cell wall layer is not formed completely and causes cell death. Flavonoids form complex compounds with extracellular proteins and soluble proteins that can damage the bacterial cell membrane and followed by the release of intracellular compounds. Saponins have a detergent-like surface, which can reduce the surface tension of the cell wall and damage the permeability of bacterial membranes.[14]

Tannin toxicity can damage bacterial cell membranes, it can shrink cell walls or cell membranes thus disrupting cell permeability. Terpenoids will react with transmembrane proteins (porins) on the outer membrane of the bacterial cell wall by forming strong polymeric bonds resulting in porin damage and impaired bacterial cell wall permeability.[15,16]

Green okra fruit extract 12.5% had an inhibition zone diameter (9.63mm), OD value (0.5280), and no *E. faecalis* colony growth. This means that the material has the ability to inhibit *E. faecalis* in the category below resistant (very weak), as evidenced by the large OD value (low bacterial density in the media), but with no subculture growth of bacterial colonies. This is because the active substance content in 12.5% green okra fruit extract is too low in inhibiting bacterial growth, but bacteria have no ability to grow after subculture, so this concentration is said to be the MBC concentration.

Green okra fruit extract 6.25% has an inhibition zone diameter (8.15 mm), OD value (0.7168), and there is a colony growth of E. faecalis of 12.4. This means that the material has the ability to *inhibit E. faecalis* category under resistant (very weak), as shown by the large OD value (low density of bacteria in the media), but after subculture there is still growth of bacterial colonies. The low content of active substances that are antibacterial at this concentration is still able to inhibit the growth of *E. faecalis* bacteria, and after subculture it still shows the ability form colonies. Therefore, 6.25% green okra fruit extract is referred to as MIC.

Based on data analysis, it appears that there are significant differences in antibacterial power between all groups of green okra fruit extract concentrations as well as NaOCl 2.5% and EDTA 17%. This is because the increasing concentration of green okra fruit extract will increase the content of active substances that are antibacterial (Polyphenols, Alkaloids, Flavonoids, saponins, tannins, steroids, terpenoids) in the material[11]. Therefore, the more the concentration increases, the higher the antibacterial ability, which can be shown by the increase in the diameter of the inhibition zone, the decrease in OD value and the reduction in colony growth or even no growth.

While NaOCl 2.5% and EDTA 17% have a very strong antibacterial power. This is because the material is a root canal irrigation material that has been used in root canal treatment, so the material has been made to meet the qualifications as an irrigation material that is antibacterial, but the antibacterial power of NaOCl is greater than EDTA. The antibacterial mechanism has been described above, while the antibacterial mechanism of EDTA is through inhibition of cation availability and can destabilize the bacterial cell membrane through the formation of divalent cation complexes that act as salt bridges between membrane micromolecules, such as lipopolysaccharides, which are found in many gram-negative bacteria.[17]

EDTA or Ethylenediaminetetraacetic acid is a chelator solution that is often used in endodontic treatment. It's an inorganic component solvent and has a lower antibacterial effect, so it's used as a complement in root canal irrigation after NaOCl. It is relatively non-toxic and slightly irritating in weak solution concentrations. The effect of EDTA on dentin depends on the concentration of the EDTA solution and the length of time it is in contact with dentin[7].

CONCLUSION

The highest phytochemical content of green okra fruit extract is polyphenols 8.11%, followed by alkaloids 6.88%, flavonoids 5.01%, saponins 4.02%, tannins 3.81%, steroids 3.22%, terpenoids 2.95%. Green okra fruit extract 100% has antimicrobial power against E. faecalis similar to NaOCl 2.5% and EDTA 17%, MIC concentration of 6.25% and MBC concentration of 12.5%.

Declaration by Authors

Ethical Approval: Not Required

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Conflict of Interest: The authors declare no conflict of interest.

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