International Journal Dental and Medical Sciences Research
Volume 6, Issue 2, Mar - Apr 2024 pp 278-283 www.ijdmsrjournal.com ISSN: 2582-6018

Antibacterial Ability of Green Okra Fruit (Abelmoschus esculentus) Extract at Concentration of 12, 5%, 25%, 50%, and 100% against Enterococcus faecalis

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Submitted: 16-03-2024 Accepted: 30-03-2024

ABSTRACT: Introduction: Enterococcus faecalis is a resistant bacterium with virulence factors found in 77% of Root Canal Treatment (RCT) failure cases. Irrigation materials commonly used in RCT are 2.5% NaOCl and 17% EDTA. 2.5% NaOCl can dissolve organic, vital pulp, and necrotic tissue, while 17% EDTA dissolves dentin and smear layer. However, 2.5% NaOCl and 17% EDTA irritate soft and periapical tissues. Green okra fruit has secondary antibacterial metabolite compounds, such as alkaloids, flavonoids, saponins, tannins, and terpenoids. This research was conducted to determine the antibacterial activity of green okra fruit extract concentrations of 12.5%, 25%, 50%, and 100% against E. faecalis. **Materials and Method:** This research uses in vitro laboratory experiment with a post-test-only control group design. The antibacterial test uses the disc diffusion method consisting of 7 research groups, which are green okra fruit extract in concentrations of 12.5%, 25%, 50%, 100%, 2.5% NaOCl, 17% EDTA, and aquadest solution (negative control). Antibacterial activity was seen from the clear zone around the disc paper. **Results:** Average diameter of the inhibitory zone of green okra fruit extract concentrations of 12.5% is 9.63 mm, 25% (14.39 mm), 50% (16.64 mm), 100% (19.28 mm), 2.5% NaOCl (24.25 mm), 17% EDTA (20.75 mm), and aquadest solution (0.00 mm). Data analysis with the Mann-Whitney test showed significant differences between all study groups ($P<0.05$). **Conclusion:** The antibacterial activity of green okra fruit extract against E. faecalis increased from concentrations of 12.5%, 25%, 50%, and 100%, but smaller than 2.5% NaOCl and 17% EDTA.

Keywords: Antibacterial test, Green okra fruit, Enterococcus faecalis

I. INTRODUCTION

Root canal damage can be caused by caries, fractures, or in- fection from the gingivalis sulcus. The most common etiol- ogy is irritants due to bacterial invasion and their products.[1] Bacterial irritant stimulation that is too strong in the pulp causes cell death, leading to pulp necrosis.[2] Only a few bacteria can survive in nutritionally restricted conditions such as pulp necrosis, including Enterococcus faecalis (E. faecalis).[3] 77% of root canal treatment failed cases are caused by reinfection due to E. faecalis resistance.[4] High resistance is caused by various virulence factors of E.faecalis, among others, having LTA (Lipoteichoic acid), which plays a role in the formation of biofilms, surface adhesions that play a role in the process of bacterial attachment in root canals, prote- olytic enzymes such as gelatinase and adhesin serine pro- tease that cause E. faecalis to survive as a single organism and resistant in root canals.[5]

Pulp necrosis can be done through Root Canal Treat- ment (RCT). Three main stages in RCT are biomechanical preparation, sterilization, and root canal obturation. The biomechanical preparation and sterilization stage is always accompanied by the root canal irrigation process.[2] Root canal irrigation aims to eliminate microorganisms, dissolve necrotic tissue, and act as a lubricant.[6] Commonly used irrigation materials are 2.5% NaOCl and 17% EDTA. Both of these ingredients are antibacterial and work synergistically. 2.5% NaOCl can dissolve organic components of dentin and biofilms, vital pulp tissue, and necrotic tissue.[7]

17% EDTA acts as a chelating agent to bind calcium ions (inorganic com- ponents) to dentin hydroxyapatite, then dentin demineraliza- tion occurs. The demineralization process reduces micro- dentin hardness so that dentin becomes softer and easier to instrument. [8] However, 2.5% NaOCl is irritative and has an unpleasant odor and taste,[9] while 17% EDTA has little antibacterial activity and cannot dissolve the organic compo- nents of dentin.[7]

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Chemical irrigation materials are toxic, so alternative irri- gation materials from natural materials are needed. One of them is the green okra fruit (Abelmoschus esculentus). Green okra fruit extract contains secondary metabolite compounds that act as antibacterials, such as alkaloids, flavonoids, saponins, tannins, and terpenoids.[10] Okra can also act as an antioxidant, anti-inflammatory, antibacterial, and has anti- cancer activity.[11] This study aimed to determine the antibacterial activity of green okra fruit extract with concentrations of 12.5%, 25%, 50%, and 100% against E. faecalis compared to 2.5% NaOCl and 17% EDTA.

II. MATERIALS AND METHOD

Ethical approval for this study (1774/UN25.8/KEPK/DL/2022) was provided by the Ethical Committee of Medical Research at the Faculty of Dentistry in Jember University, Jember, on September 05, 2022. This type of research is an in vitro experimental laboratory with a post-test-only control group design. The study consisted of seven groups: green okra fruit extract concentrations of 12.5%, 25%, 50%, 100%, 2.5% NaOCl, 17% EDTA, and aquadest solution (negative control). Each study group was carried out five repetitions.

Tools

Non-insulated petridish 100mm (Pyrex, France), disc paper (No Brand), blender (Philips, Holland), test tube (Iwaki, Japan), Erlenmeyer tube (Schott Duran, Germany), incubator (Espec, Japan), micropipette (Socorex, Switzerland), autoclave (Hirayama, Japan), caliper (No Brand).

Materials

Green okra fruit extract, E. faecalis ATCC 29212, Mueller Hinton Agar (MHA) (Oxoid, UK), Mueller Hinton Broth (MHB) (Oxoid, UK), Aquadest solution (No Brand), 96% ethanol (No Brand), 2.5% NaOCl (Onemed, Indonesia), 17% EDTA (Onemed, Indonesia).

Preparation of green okra fruit extract

Green okra fruit was obtained from PT. Mitratani Dua Tujuh Jember, later identified at the Plant Laboratory, Jember State Polytechnic. Green okra fruit was washed and dried at room temperature and avoided from the direct sunlight. The dried fruit was mashed using a blender until it becomes powder and weighed to 627 g. Green okra fruit was extracted with the maceration method using 96% ethanol solvent in a 1:2 ratio for 3×24 hours. The maceration results were concentrated at 60^{O}C for 3 hours in a rotary evaporator.

Preparation concentration of green okra fruit extract

The extract concentration obtained from the extraction process is 100%, then serial dilution was carried out to obtain concentration variations of 50%, 25%, and 12.5%. The extract was concentrated by placing green okra fruit extract in a test tube containing 1 mL of aquadest sterile and homogenizing it with the vortex.

Inoculum suspension

The bacterial suspension was manufactured by taking one ose of E. faecalis from stock and inserting it into a test tube containing 2 ml MHB. Test tubes were incubated at 37^{o}C for 24 hours. After incubation, E. faecalis bacteria were homogenized using a vortex for 30 seconds and adjusted for turbidity to a standard of 0.5 McFarland (1.5x10^{8} CFU/ml).

Antibacterial test

The disc diffusion method is used for the antibacterial test. The suspension of E. faecalis bacteria was inoculated on Mueller Hinton Agar (MHA) media using a sterile cotton swab. Each disc paper was dripped with ten microliters of green okra fruit extract concentrations of 12.5%, 25%, 50%, 100%, 2.5% NaOCl, 17% EDTA, and aquadest solution. Paper discs were laid with a slight press on the surface of MHA media using sterile tweezers. Then, incubated in an incubator at 37^{o}C for 24 hours. The antibacterial activity can be seen by calculating the diameter of the inhibitory zone formed around the paper disc using a caliper.

Statistical analysis

The acquired data were checked for normality with Shapiro- Wilk (P>0.05) and homogeneity with Levene Test (P<0.05). The Kruskal-Wallis non-parametric statistical test was employed for all treatment groups, followed by the Mann- Whitney test (P<0.05).

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III. RESULTS

The antibacterial activity of green okra fruit extract against

E. faecalis can be seen by calculating the diameter of the inhibitory zone in the form of clear regions formed around the paper disc [Figure 1].

The diameter of the inhibitory zone of the green okra fruit extract increases in line with the increase in concentration. Negative control of

aquadest solution has no inhibitory zone (0.00 mm). There was an increase in the average diameter of the inhibitory zone of green okra fruit extract from concentrations of 12.5% (9.63 mm), 25% (14.39 mm), 50% (16.64 mm), 100% (19.28 mm), 17%

EDTA (20.75 mm), and 2.5% NaOCl (24.25 mm)[Table 1].

Data analysis started with the Shapiro-Wilk normality test. The test results obtained a significance value of P>0.05, meaning the data is normally distributed. Furthermore, the homogeneity test of the Levene Test was carried out to test the variety of populations. The homogeneity test results showed a significance value of
0.044 (P< 0.05), which means the data is inhomogeneous. The following analysis was the Kruskal-Wallis non- parametric statistical test to determine the study group's antibacterial activity difference. The results of the Kruskal- Wallis test showed a significance value of 0.000 (P<0.05), which means there is a difference in antibacterial activity in the research group. The statistical test was followed by the Mann-Whitney test to determine the significant differences between research groups. The results of the Mann-Whitney test showed significant differences between all study groups (P<0.05) [Table 2].

IV. DISCUSSION

The results showed an increase in the diameter of the inhibitory zone of green okra fruit extract sequentially from concentrations of 12.5%, 25%, 50%, and 100%. It means the higher the levels of the extract, the more active compounds contained in it. As a result, the diameter of the formed inhibitory zone becomes even larger.[12]

The results showed that the diameter of the inhibitory zone of green okra fruit extract concentration of 12.5% was in the medium category, 25%, 50%, and 100% concentration in the strong category, 2.5% NaOCl and 17% EDTA in the very strong category. In contrast, the aquadest solution was in the weak category.[13] There was an increase in the diameter of the inhibitory zone from low concentration to high concentration.

However, the size of the inhibitory zone of green okra fruit extract was still smaller than 2.5% NaOCl and 17% EDTA.

Green okra fruit extracts are still crude extracts. The active antibacterial compounds contained in the extract have not been separated purely. In addition, the extract process in this study used ethanol solvents so that there may be other components (chlorophyll, carotenoids, anthocyanins, resins) that interfere with the stability of the physical properties of the extract that affects the decrease in the effectiveness of the antibacterial activity.[14]

The diameter of the NaOCl inhibitory zone of 2.5% (24.25 mm) is related to the content of chemical elements possessed, namely sodium, oxide, and chlorine, through three mechanism stages (saponification, neutralization, and chloramination). On contact with water, NaOCl ionizes to Na^+ and OCl^- and forms Sodium Hydroxide (NaOH) and Hypochlorous acid (HOCl).[15]

The saponification reaction involves NaOH by breaking down fatty acids (phospholipids) on bacterial cell membranes into fatty acid salts and glycerol. This reaction causes damage to the cell membrane of bacteria. Neutralization reactions occur when NaOH reacts with amino acids to form water and salts. This reaction decreases pH by releasing hydroxyl ions, resulting in the denaturation of membrane proteins. A chloramination reaction occurs when hypochlorous acid (HOCl) comes into contact with organic tissue (amino acids). This reaction releases chlorine which forms chloramine after joining amino protein groups. Chlorine can inhibit bacterial enzymes leading to the irreversible oxidation of SH groups (sulphydryl groups) of essential bacterial enzymes.[15]

The diameter of the EDTA inhibitory zone of 17% (20.75 mm) is related to its ability as a chelating agent to bind Ca^{2+} and Mg^{2+} cations to the outer membrane of bacteria.[6] The cation connects lipopolysaccharide (LPS) onto the cell membrane of Gram-negative bacteria, while Gram-positive bacteria connect teichoic acid. As a result, the stability of bacterial cell membranes becomes disrupted.[16] 17% EDTA can bind Ca^{2+} ions to dentin hydroxyapatite then dentin demineralizing occurs. The demineralization process reduces microdentin hardness so that dentin becomes softer and easier to instrument.[8]

2.5% NaOCl and 17% EDTA are stronger in inhibiting E. faecalis than green okra fruit extract because the irrigation material has gone through various stages related to killing bacteria. The antibacterial activity of NaOCl 2.5% is stronger than 17% of EDTA because 2.5% NaOCl has

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Volume 6, Issue 2, Mar - Apr 2024 pp 278-283 www.ijdmsrjournal.com ISSN: 2582-6018

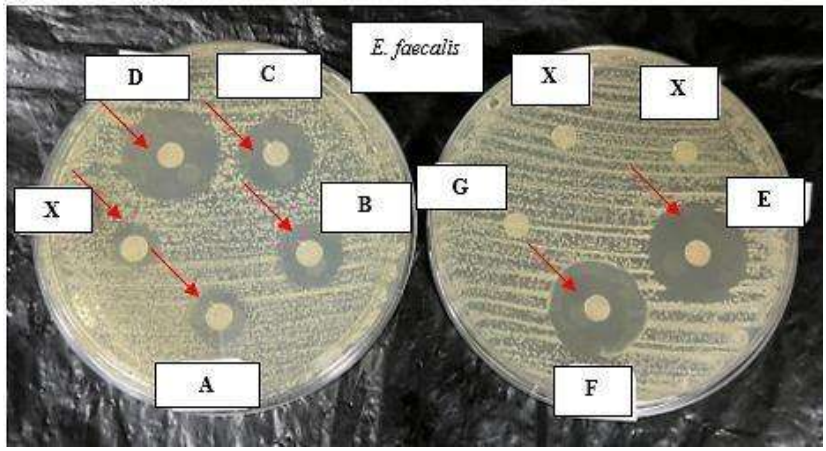

Figure 1. The inhibitory zone of green okra fruit extract, 2.5% NaOCl, 17% EDTA, and aquadest solution againstE. faecalis in the form of a clear zone around the disc paper is indicated by a red arrow. A) Green okra fruit extract inhibitory zone 12.5% concentration, B) Green okra fruit extract inhibitory zone 25% concentration, C) Green okra fruit extract inhibitory zone 50% concentration, D) Green okra fruit extract inhibitory zone 100% concentration, E) Comparator group inhibitory zone 1 (2.5% NaOCl), F) Comparator group inhibitory zone 2 (17% EDTA), G) Negative control (aquadest solution), X) Concentrations that are not used as samples.

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

Research Group	Inhibitory Zone Diameter (mm) Repetition					Average ± standard deviation
	I	II	III	IV	V	
K (-)	0	0	0	0	0	**0.00±0.00**
Extract 12.5%	9.40	9.80	10.20	9.55	9.20	**9.63±0.38**
Extract 25%	15.40	13.20	15.35	14.40	13.60	**14.39±0.99**
Extract 50%	17.60	16.40	17.40	15.60	16.20	**16.64±0.84**
Extract 100%	19.20	20.80	19.20	18.40	18.80	**19.28±0.91**
2.5% NaOCl	25.20	23.40	24.20	24.05	24.40	**24.25±0.65**
17% EDTA	21.40	21.00	20.95	20.00	20.40	**20.75±0.55**

Table 2. Mann-Whitney test results

Group	K(-)	K1	K2	K3	K4	KP1	KP2
K(-)		0.005*	0.005*	0.005*	0.005*	0.005*	0.005*
K1			0.009*	0.009*	0.009*	0.009*	0.009*
K2				0.009*	0.009*	0.009*	0.009*
K3					0.009*	0.009*	0.009*
K4						0.009*	0.028*
KP1							0.009*
KP2							

* = There are significant differences between groups

Lestari , Meilawaty and Asri broad-spectrum antibacterial activity, while 17% EDTA more specifically kills Gram-negative bacteria.[9]

The component of secondary metabolites of green okra fruit extract is antibacterial in the form of alkaloids, flavonoids, saponins, tannins, and terpenoids.[17] Flavonoids inhibit nucleic acid synthesis by damaging DNA-gyrase, which plays a role in transcription and DNA replication.[18]

Tannins interfere with polypeptide components resulting in incomplete cell wall formation.[19] Saponins decrease the cell wall's surface tension, increasing cell permeability, which triggers cell leakage.[20] Terpenoids damage cell membranes through the process of membrane breakdown by lipophilic components.[19] Alkaloids inhibit the formation of peptidoglycan, so the cell wall becomes incomplete.[21]

The antibacterial activity of NaOCl 2.5% and EDTA 17% are stronger than green okra fruit extract. However, 2.5% NaOCl and 17% EDTA are irritative when in contact with soft tissue and periapical tissue, and trigger inflammatory reactions that cause pain, mucosal ulceration, and swelling in tissue[22], so their use is limited to certain concentrations.[9]

V. CONCLUSION

Based on the results of the study, it was concluded that the antibacterial activity of green okra fruit extract (Abelmosuchus esculentus) increased from concentrations of 12.5%, 25%, 50%, and 100%, but still smaller than 2.5% NaOCl and 17% EDTA against Enterococcus faecalis.

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