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The Inhibition of Green Okra Fruit (Abelmoschus Esculentus) Extract against Streptococcus Viridans Root Canals of Teeth

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ABSTRACT

Background: Streptococcus viridans is the most frequently isolated bacterium from infected root canals (63%) and the leading cause of life-threatening sub-acute endocarditis (35%). The use of 2.5% NaOCl as a root canal irrigation material has disadvantages, it is a bad smell and taste, causing allergies, irritating and toxic, so other alternatives are needed. The green okra fruit extract has secondary antibacterial metabolites such as flavonoids, alkaloids, saponins, tannins, and terpenoids. **Purpose:** This study aims to determine the inhibition of green okra fruit extract against S. viridans. **Methods:** This research is an experimental laboratory in vitro with a post-test-only control group design. Antibacterial test using the disk diffusion method which consisted of 5 research groups (green okra extract with a concentration of 1,56%, 3,125%, 6,25%, 12,5%, and 2,5% NaOCl) and 4 repetitions. The result was analyzed using non-parametric statistical tests.

Results: The average diameter of inhibition zone of each concentration of green okra fruit extract is 1,56% (0 mm), 3,125% (0 mm), 6,25% (14,58 mm), 12,5% (18,19 mm), and 2,5% NaOCl (23,55 mm). The Kruskal-Wallis test showed a significance value of less than 0.05 which meant that there was a difference in inhibition in all groups. The Mann-Whitney test showed that there were significant differences between the study groups (α<0,05), except for the green okra extract with a concentration of 1,56% with 3,125%.

Conclusion: Green okra fruit extract (Abelmoschus esculentus) has inhibition against S. viridans at concentrations of 6,25% (14,58 mm) and 12,5% (18,19 mm) including the strong category.

KEY WORDS: a disk diffusion, green okra fruit extract, inhibition, Streptococcus viridans

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INTRODUCTION

The Global Burden Disease (2016) states that 3,58 billion people or half the world's population experience dental and oral health problems, especially caries[1]. Untreated Caries for a long time will result in pulp infection which will cause necrosis of the pulp. Pulp necrosis is the partial or complete death of pulp tissue which can be caused by physical trauma (mechanical and thermal), chemical irritation, and bacteria[2]. Bacteria are the main etiology of pulp necrosis. The most dominant bacteria in root canal infections are gram-positive anaerobes (75%) such as streptococcus (28%), staphylococcus (15%), and, corynebacteria (10-25%). Streptococcus viridans (S. viridans) is an oral bacterium belonging to the α-hemolytic or non-hemolytic streptococcus group that is most frequently isolated from infected root canals (63%) and the leading cause of life-threatening sub-acute endocarditis (35%)[3]. The treatment for pulp necrosis is root canal treatment. Root canal treatment is a procedure performed by removing vital or necrotic pulp tissue to eliminate the bacterial infection, accelerate healing and maintain teeth as long as possible in the oral cavity. There are three main stages in root canal treatment, namely biomechanical preparation, sterilization, and obturation. Biomechanical preparation is done by opening access to the pulp chamber, shaping, and cleaning the root canal with an irrigation solution. Irrigation is one of the important stages in root canal treatment because it can determine the success of treatment[4,5]. Root canal irrigation materials are often used from chemicals (synthetic) including sodium hypochlorite (NaOCl) 2.5%[5]. NaOCl can dissolve necrotic tissue, dentin,

and pulp. NaOCl is biocompatible and has antibacterial properties through three reactions that are saponification, chloramination, and neutralization so it has a broad spectrum of action[3,5]. The use of 2,5% NaOCl as a root canal irrigation material has disadvantages, it is a bad smell and taste, causes allergies, irritates when enters the periapical tissues, and is toxic when used in excessive concentrations[4,5]. To overcome the disadvantage of synthetic root canal irrigation materials, alternatives from herbal plants are needed because they are affordable, easy to obtain, and have minimal side effects, making them safer to use. One of the plants that can be used is okra (Abelmoschus esculentus).

The okra plant is one of the best pharmacological sources because it has several important biological activities such as antioxidant, anti-inflammatory, antibacterial, anticancer, and anti-diabetic[6]. There are two varieties of okra cultivated in Indonesia, namely red okra and green okra. Green okra cultivation is very easy to do in many places with various soil types and pH, such as Ngampel, Kendal, Boja, Banten, and Jember. The production of green okra fruit in Jember is quite high (1,500 tons per year) and has penetrated the export market to Japan[7]. Green okra fruit can be used as a vegetable (fried, steamed, boiled), as a drink, and as a flavoring agent[8]. Utilization of green okra fruit in dentistry is still rare. Based on the phytochemical test, green okra fruit extract contains secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and terpenoids which are antibacterials so expected to inhibit the growth of S. viridans[9].

This study aims to determine the inhibition of green okra fruit extract (Abelmoschus esculentus) against S. viridans and the concentration of green okra fruit extract (Abelmoschus esculentus) which can inhibit the growth of S. viridans. The expected benefit is to provide information about the benefits of green okra fruit extract (Abelmoschus esculentus) as an antibacterial against S. viridans that causes root canal infections, as a preliminary study in the development of green okra fruit as an alternative root canal irrigation material and the results of the research can be used as a reference for further research.

METHODS

Equipment

Petri dish (Pyrex, Japan), test tubes (Pyrex, Japan), Erlenmeyer flask (Pyrex, Jerman), stir bar/spatula, analytical balance (Ohaus, Germany), macerator, sieve, mask (OneMed, Indonesia), handscoon (OneMed, Indonesia), funnel glass, bunsen, blender (Cosmos, Indonesia), autoclave (Hadshin Medical Co., LTD), inoculating loop, measuring cylinder, incubator (Binder BD 53), desiccator, caliper (Medesy, Italy), laminar flow, electric stove, cotton swab, test tubes rack, tube, stopwatch, rotary vacuum evaporator (Heidolph, Jerman).

Material

Green okra fruit (Abelmoschus esculentus), 70% ethanol, aquades (Aqua Pro injection), sodium hypochlorite ((NaOCl) 2,5% (OneMed, Indonesia), S. viridans (Laboratorium Research Centre, RSGM Unair), 0.5 Mc Farland standard solution, paper disk, cotton, filter powder, BHI-A (Merck, Germany), BHI-B (Merck, Germany), label paper (Kenjoy, Indonesia), aluminium foil.

Okra Fruit Extract

This type of research was an experimental laboratory (in vitro) using a post-test-only control group design. Ripe green okra fruit (dark green) and seeds, 10-13 cm, fresh (not wilted) obtained from PT. Mitratani Dua Tujuh Jember was identified then washed, cut into small pieces, and dried in a place protected from direct sunlight and in the oven at 50°C for 3 days. Green okra fruit simplicia was crushed using a blender, sifted, and weighed (374,5 grams). Green okra fruit powder was macerated using 70% ethanol in a ratio of 1:5 for 3 days and diluted (serial dilution method) to obtain a concentration of 1,56%; 3,125%; 6,25%, and 12,5%.

Antibacterial Test

Antibacterial test using disk diffusion method. The bacterial suspension was taken by cotton swab and then inoculated on BHI-A media and leveled by streaking movements throughout the plate surface. A paper disc is soaked in green okra fruit extract until all parts are wetted for about 30 seconds then placed on the media using tweezers carefully with a little pressure. The Petri dish is closed and then put into the desiccator in an inverted position so that bacterial growth is not disturbed (minimizing the fall of moisture into the media). The desiccator was put into the incubator for 1x24 hours at a temperature of 37°C. Inhibitory power is seen by measuring the inhibition zone formed around the disc paper using a vernier caliper.

Data Analysis

The average diameter of the inhibition zone was statistically analyzed using SPSS 25 through normality, homogeneity, Kruskal Wallis, and Mann-Whitney tests. The normality test used was the Shapiro Wilk because the number of samples was less than 50. This test was conducted to determine whether the data distribution was normal. Based on the Shapiro-Wilk normality test results, a significance value of $\alpha > 0,05$ means that the data is normally distributed. Then a variant homogeneity test was carried out using the Levene test to test the population variance. The significance value obtained from this test is 0,00 ($\alpha<0,05$) meaning that the data is not homogeneous.

Furthermore, the non-parametric statistical test was carried out by Kruskal Wallis because the data was normally distributed but not homogeneous. The results of this test showed a significance value of less than 0,05 (0,00) meaning that there were differences in inhibition in all groups. The

Mann-Whitney follow-up test needs to be carried out to determine the significance of the differences in each treatment group. The Mann-Whitney test results showed significant differences between research groups (α<0,05), except for the green okra fruit extract research group with a concentration of 1,56% and 3,125%.

RESULT

The results of the research on the inhibition of green okra fruit extract (Abelmoschus esculentus) on S. viridans were determined by measuring the clear zone (inhibition) formed around the disc paper. The inhibition zone can be shown in the image below

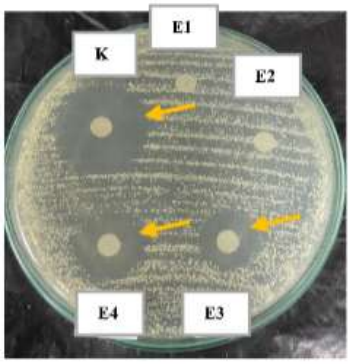

Figure 1. The inhibition zone of green okra fruit extract around the disc paper is indicated by a yellow arrow. E1) green okra fruit extract concentration 1,56% E2) green okra fruit extract concentration 3,125% E3) green okra fruit extract concentration 6,25% E4) green okra fruit extract concentration 12,5% K) control (NaOCl) 2, 5%

Figure 1. shows that of the various concentrations of the green okra fruit extract, which did not have an inhibition zone were green okra fruit extract concentrations of 1,56% (E1) and 3,125% (E2). The average diameter of inhibition zone and the standard deviation of each concentration of green okra fruit extract and 2,5% NaOCl can be seen clearly in the average difference in table 1 below.

Table 1. The Average Diameter of Inhibition Zone and Standard Deviation

Group	N	$\bar{X} \pm$ SD (mm)
E1	4	0 ± 0,00
E2	4	0 ± 0,00
E3	4	14,58 ± 0,653
E4	4	18,19 ± 0,459
K	4	23,55 ± 0,499

Keterangan:
N = Number of repetitions
$\bar{X}$ = The average diameter of inhibition zone
SD =Standard deviation

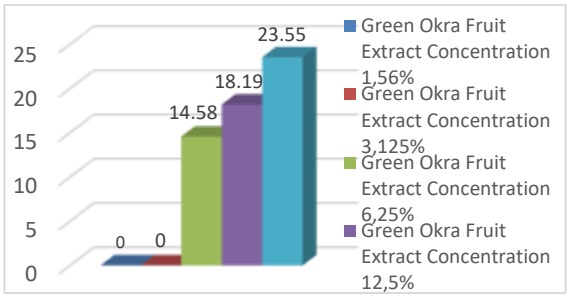

Figure 2. Bar chart of the average diameter of inhibition zone

Based on the table and bar chart above, it appears that the average diameter of the inhibition zone experienced an increase in diameter values starting from green okra fruit extract concentration of 6.25% (E3) (14.58 ± 0.653 mm), green okra fruit extract concentration of 12.5% (E4) (18.19 ± 0.459 mm), and the largest was the control group NaOCl 2.5% (K) (23.55 ± 0.499 mm).

DISCUSSION

The results showed that green okra fruit extract concentrations of 1,56% and 3,125% had no inhibition

because of the absence of clear zones around paper discs (figure 1). This is probably due to either the content of secondary metabolites in the extract being too small or there was no diffusion in the agar media, so it could not inhibit the growth of S. viridans[10]. The concentration of green okra fruit extract which inhibited the growth of S. viridans was 6,25% (14,58 mm) and 12,5% (18,19 mm) (table 1). Extracts with higher concentrations will form a larger inhibition zone and the more concentrated extract, the more secondary metabolites in it (figure 2)[11]. The content of secondary metabolites in green okra fruit extracts such as flavonoids, alkaloids, saponins, tannins, and terpenoids are antibacterial so they can inhibit the growth of S. viridans[9].

The flavonoid quercetin (60-75%) in okra has several antibacterial mechanisms. The hydroxyl group of flavonoids (polar) can bind to the peptidoglycan layer of the bacterial cell wall like lipids and amino acids (polar) and cause damage to the cell wall. Damage to the bacterial cell wall allows flavonoids to enter the cell nucleus and come into contact with DNA so that the DNA lipid structure is damaged (bacterial lysis). Flavonoids inhibit the function of cell membranes by changing the fluidity of hydrophobic and hydrophilic areas, disrupting membrane permeability, and inhibiting ATPase and phospholipase binding[12]. Flavonoids inhibit nucleic acids by binding to Gyrase B (a subunit of the gyrase enzyme) thereby interfering with bacterial DNA replication[13]. Flavonoids are also able to inhibit efflux pumps, bacterial toxins, and bacterial cell wall synthesis[14].

Saponins in green okra fruit have characteristics like foam when reacted with water and shaken (detergent). The structure of saponins consists of a steroid or triterpenoid aglycone (non-polar) which is bound to one or more oligosaccharide groups such as pentoses, hexoses, or uronic acid (polar) so that they have a strong active surface against bacteria. Saponins are antibacterial through a saponification reaction, namely the reaction between fat or triglycerides and alkali (NaOH and KOH) which can reduce the surface tension of the bacterial cell wall and increase the permeability of the cell membrane[12,15]. The antibacterial ability of alkaloids is to inhibit the topoisomerase enzyme's work, interfering with bacterial DNA replication and inhibiting its growth. Alkaloids can also interfere with cell wall synthesis by binding to peptidoglycan constituent components so that the cell wall does not form completely and suffers death due to lysis.

The other metabolites in green okra fruit are tannins and terpenoids. Tannins are part of polyphenolic compounds that are capable of complexing with proteins, polysaccharides, and nucleic acids, causing damage to bacterial cells. Tannins can also interfere with cell permeability by shrinking walls or membranes so that cells cannot carry out their life activities, inhibit their growth, or die. The mechanism of terpenoids against bacteria is by damaging the cell wall layer. Terpenoids can agglomerate proteins resulting in disturbances in the osmotic pressure of bacterial cells[16,17].

Sodium hypochlorite (NaOCl 2.5%) has a very strong (23,55 mm) inhibition against the growth of S. viridans (table 1). This is possibly due to the reaction between NaOCl and water (H_2O) to form hypochlorous acid (HOCl) and sodium hydroxide (NaOH), causing potential reactions with other molecules (saponification, chloramination, and neutralization)[18]. The following is the reaction of NaOCl with water:

$$NaOCl + H_2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^+ + OH^- + OCl^-$$

The difference in the diameter of the inhibition zone between 2,5% NaOCl (23,55 mm) and green okra fruit extract concentrations of 6,25% (14,58 mm) and 12,5% (18,19 mm) indicates that both have different abilities in inhibiting S. viridans (table 1). According to Davis and Stout, the inhibition strength of a material is categorized into four namely weak categories (≤ 5 mm), medium categories (5-10 mm), strong categories (10-20 mm), and very strong categories (> 21 mm)[19]. Based on these criteria, the inhibition of green okra fruit extract at concentrations of 6,25% and 12,5% is a strong category, while the inhibition of 2,5% NaOCl is a very strong category.

Secondary metabolite compounds in green okra fruit extract and NaOCl have a similar mechanism as an antibacterial, there are inhibiting cell walls, cell membranes, and DNA. Whole extract of green okra fruit obtained using organic solvents (ethanol) still contains various unwanted compounds such as dyes (chlorophyll a and b, carotenoids, anthocyanins), carbohydrates, waxes, resins, etc which are often detrimental (disturbing the stability of the physical properties of the extract) and reduce the levels of active compounds in it)[20]. The use of a whole extract of green okra fruit allows the formation of antibacterial power which is smaller than 2,5% NaOCl which has been standardized through various stages in its function as an antibacterial and consists of only a few chemical elements like sodium, oxide, and chlorine so that it has high antibacterial power[21]. Green okra fruit has the potential as an alternative root canal irrigation material, but still requires a lot of further research.

CONCLUSION

Based on the results of the study it can be concluded that green okra fruit extract (Abelmoschus esculentus) has an inhibitory effect on S. viridans at a concentration of 6,25% (14,58 mm) and 12,5% (18,19 mm) which is included in the strong category.

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