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Minimum Bactericidal Concentration (MBC) of Green Okra Fruit Extract (*Abelmoschus esculentus*) against *Streptococcus mitis*

Fatin Saifana Adila[1], Sri Lestari[2], Dwi Warna Aju Fatmawati[3]

[1]Faculty of Dentistry, University of Jember, Indonesia,
[2]Department of Conservation, Faculty of Dentistry, University of Jember, Indonesia,
[3]Department of Conservation, Faculty of Dentistry, University of Jember, Indonesia

Corresponding Author: Sri Lestari

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ABSTRACT

Streptococcus mitis (*S.mitis*) is a bacterium commonly found in infected root canals, which necessitate root canal treatment (RCT). Irrigation is an important stage to eliminate the root canal from debris and microorganism. Sodium hypochlorite (NaOCl) 2,5% and Ethylenediamine tetraacetic acid (EDTA) 17% are frequently used as irrigation agents. NaOCl can be toxic to periapical tissues, whereas EDTA exhibits low antibacterial properties. The use of natural materials such as green okra fruit is considered safer than chemical agents. Green okra fruit extract possesses antibacterial properties, a requirement for an ideal irrigation material. This study aims to determine the minimum bactericidal concentration (MBC) of green okra fruit extract against *S.mitis*. This type of research was a laboratory experimental study with a post-test only control group design. There are six treatment groups: green okra fruit extract concentrations of 6.25%, 12.5%, 25%, NaOCl 2.5%, EDTA 17%, and aquadest. The MBC test using the solid dilution method and determined by observing the growth of *S.mitis* colonies on Petri dishes. The research results were analyzed using the non-parametric Kruskal-Wallis and Mann-Whitney tests. Bacterial colonies were observed in extract concentrations of 6.25% (36 colonies), 12.5% (12 colonies), and aquadest (159 colonies). No bacterial colony growth was detected at extract concentration of 25%, NaOCl 2.5%, and EDTA 17%. The Kruskal-Wallis test showed a significance value of 0.00 ($\alpha<0.05$). The Mann-Whitney test indicated significant differences among all research groups, except between the green okra fruit extract concentration of 25%, NaOCl 2.5%, and EDTA 17% groups. The MBC of green okra fruit extract against *S.mitis* bacteria is not at 12.5% but at 25%. The 25% concentration exhibits properties similar to NaOCl 2.5% and EDTA 17%, as evidenced by the absence of *S.mitis* colony growth.

Keywords: Green okra fruit extract, minimum bactericidal concentration (MBC), Streptococcus mitis

INTRODUCTION

Dental caries is a multifactorial disease characterized by the demineralization of the tooth's hard tissues. One of the contributing factors to dental caries is the presence of bacterial biofilm.[1] Bacteria can invade and cause caries to spread, penetrating the root canal and causing infection if not treated promptly. *Streptococcus mitis* (*S.mitis*) was one of the most common microorganisms (53.3%) isolated in root canal infections.[2] These bacteria can be spread and causing

invasive infections such as endocarditis, especially in individuals with weakened immune systems. [3,4]

Two essential elements in the fundamental principles of endodontics are cleaning and shaping. Achieving adequate cleanliness involves the use of mechanical instrumentation combined with endodontic irrigation to lubricate the root canal system, dissolve organic tissue, and most importantly, clean and disinfect the root canal.[5] Irrigants solutions such as 2.5% sodium hypochlorite (NaOCl) combined with 17% ethylenediamine tetraacetic acid (EDTA) are commonly used. The combination of 2.5% NaOCl and 17% EDTA irrigation materials is considered more efficient in reducing bacterial growth and preventing biofilm formation.[6] NaOCl 2.5% has a broad antimicrobial spectrum, but it causes irritation if it penetrates the periapical tissue. A case report by Faras *et al*. (2016) showed that 2.6% NaOCl caused a patient to experience burns in the right infraorbital area due to NaOCl extrusion during root canal treatment.[7] The use of 17% EDTA also has a disadvantage, which is its lower antibacterial effect compared to 2.5% NaOCl. An ideal irrigant should have strong antimicrobial action against a broad microorganism spectrum and low toxicity.[8]

Okra (*Abelmoschus esculentus*) is a vegetable that is widely cultivated in Jember.[9] Okra is a multifunctional plant, it is have many bioactivities such as cardioprotective, renal, neuroprotective, anti-fatigue, anti-cancer, and antibacterial.[10] The okra fruit contains antibacterial properties, making it a potential alternative material for root canal irrigation. The antibacterial properties of okra come from alkaloids, flavonoids, saponins, tannins, and terpenoids.[10,11] Antibacterial properties can be classified into bacteriostatic and bactericidal. Bacteriostatic is antibiotics that inhibits the bacterial growth but does not kill the bacteria whereas bactericidal is antibiotic that kills the bacteria.[12] The bactericidal property of an antibacterial agent is determined through a Minimum Bactericidal Concentration (MBC) test. The MBC test is a continuation of broth dilution testing after incubation and determination of antimicrobial agent's Minimum Bacteriostatic Concentration (MIC). MIC refers to the lowest concentration of an antibiotic that prevents visible growth of a microorganism following 16–20 hours of incubation.[13] The MBC is defined as the lowest concentration that is capable to killing 99.9% of the tested bacteria, MBC is a more reliable measure of antibiotic activity.[14]

Previous research has shown that the antibacterial effect of green okra fruit extract against *S.mitis* is present at a concentration of 6.25%, suggesting that the MBC of green okra fruit extract against *S.mitis* might be at a concentration higher than 6.25%.[15] This is supported by the findings of Lestari et al.(2024), which demonstrated that the MIC of okra extract against *Enterococcus faecalis* was 6.25%, while the MBC was 12.5%.[11] The aim of this study is to determine the MBC of green okra fruit extract against *S.mitis* by testing concentrations of 6.25%, 12.5%, and 25%.

MATERIALS & METHODS

This research is an in vitro laboratory experiment with a post-test-only control group design. The study was conducted from November 2023 to January 2024. Green okra fruit samples were obtained from PT. Mitra Tani Dua Tujuh, Jember, which had been identified in the Plant Laboratory, Department of Agricultural. Production, State Polytechnic of Jember. The bacteria for this study were obtained from the Research Center Laboratory, Faculty of Dentistry, Airlangga University. The study used six samples consisting of green okra fruit extract concentrations of 6.25%, 12.5%, 25%, NaOCl 2.5%, EDTA 17%, and aquadest. The sample size was calculated using the Federer formula, and each of the six samples carried out four repetitions.

The extract was prepared at the Bioscience Laboratory, Faculty of Dentistry, University of Jember. Okra fruits were washed, thinly sliced, and dried using the air-drying method

away from direct sunlight for 12 days. The dried okra fruits then ground and sieved through a 40-mesh sieve. The Simplicia extracted using the maceration method with 96% ethanol solvent for 3x24 hours. The maceration extract was filtered and evaporated for 24 hours at 50ºC, resulting in the extraction of 100% green okra fruit extract concentration.

The next stage of the research involves preparing Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA), preparing *S.mitis* suspensions, and MBC tests carried out at the Research Center Laboratory, Faculty of Dentistry, Airlangga University. The *S.mitis* suspensions are prepared by taking a single colony of *S.mitis* bacteria and inoculating it into a test tube containing 5 ml of MHB. The test tubes are incubated for 24 hours at 37ºC. The bacterial culture is adjusted to the McFarland turbidity standard of 0.5 (1.5 x 10 CFU/ml).

The MBC test was conducted using the broth dilution method after performing the MIC test using the broth dilution method. Before conducting the MBC test, the extract was diluted using serial dilution with MHB diluent to obtain green okra fruit extract concentrations of 25%, 12.5%, and 6.25%. *S.mitis* bacteria were inoculated into the test tubes of all research groups and incubated for 24 hours at 37ºC. A total of 0.1 ml of the bacterial culture from each test tube was taken and spread onto MHA plates using the spread plate method. The plates were then reincubated for another 24 hours at 37ºC. Plates showing no colony growth or colonies ≤0.1% of the number of colonies in the original inoculum (aquadest) were determined as the MBC values.[16] Colony counts were conducted by three researchers manually, counting visible colony growth.

Statistical Analysis

The research data were analyzed using SPSS version 26. Normality was tested using the Shapiro-Wilk test, followed by homogeneity testing using Levene's test. The results indicated that the data were normally distributed but not homogeneous, so further analysis was conducted using the non-parametric Kruskal-Wallis test and Mann-Whitney test.[17]

RESULT

The results of the MBC test of green okra fruit extract *(Abelmoschus esculentus)* against *S.mitis* bacteria are presented as colony growth in Petri dishes, as shown in Figure 1.

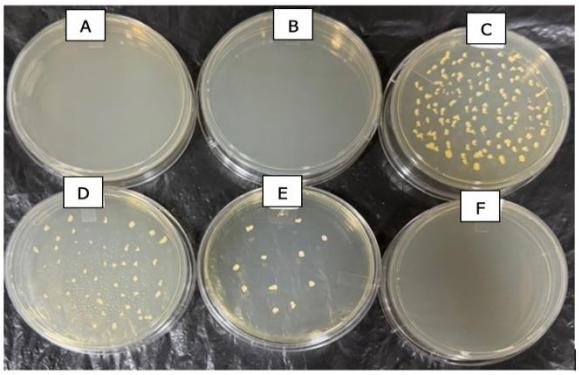

Figure 1. The growth of *S. mitis* colonies on MHA solid media. A) Colony growth on positive control 1 (2.5% NaOCl), B) Colony growth on positive control 2 (17% EDTA), C) Colony growth on negative control (aquadest), D) Colony growth at extract concentration of 6.25%, E) Colony growth at extract concentration of 12.5%, F) Colony growth at extract concentration of 25%.

Colony growth decreases as the concentration of green okra fruit extract used increases. The highest average colony growth of *S.mitis* is found in K- followed by green okra fruit extract concentrations of 6.25% and 12.5%, while NaOCl 2.5%, EDTA 17%, and green okra fruit extract concentration of 25% show no growth of *S.mitis* colonies. The MBC is the lowest concentration that can kill 99.9% or at most

have 0.1% of test bacterial colonies compared to the number of colonies in the original inoculum. Green okra fruit extract concentration of 25% shows no colony growth (colonies ≤ 0), thus it can be established as the MBC value in this study. The average number of *S.mitis* colonies is shown in Table 1.

Table 1. Average growth of *S. mitis* bacterial colonies

Research group	Average
Extract 6.25%	36
Extract 12.5%	12
Extract 25%	0
NaOCl 2.5% (K1+)	0
EDTA 17% (K2+)	0
Aquadest (K-)	159

The Shapiro-Wilk test results indicated normally distributed data ($\alpha > 0.05$). The Levene's test for homogeneity showed a significance value of 0.00 ($\alpha < 0.05$), indicating non-homogeneous data. Data analysis proceeded using non-parametric Kruskal-Wallis and Mann-Whitney tests. The Kruskal-Wallis test showed a significance value of 0.00 ($\alpha < 0.05$), indicating differences among research groups. Subsequently, the Mann-Whitney test was conducted to identify significant differences between research groups. A significance level of $\alpha < 0.05$ would indicate such differences. Detailed results of the Mann-Whitney test are presented in Table 2.

Table 2. The result of Mann Whitney test

Research Group	Extract 6,25%	Extract 12,5%	Extract 25%	NaOCl2,5% (K1+)	EDTA17% (K2+)	aquadest (K-)
Extract 6,25%		0,021*	0,014*	0,014*	0,014*	0,021*
Extract 12,5%	0,021*		0.014*	0,014*	0,014*	0,021*
Extract 25%	0,014*	0,014*		1,000	1,000	0,014*
NaOCl 2,5% (K1+)	0,014*	0,014*	1,000		1,000	0,014*
EDTA 17% (K2+)	0,014*	0,014*	1,000	1,000		0,014*
aquadest(K-)	0,021*	0,021*	0,014*	0,014*	0,014*	

Based on Table 2, there are significant differences between the research groups except for NaOCl 2.5% with EDTA 17%, NaOCl 2.5% with green okra fruit extraxt concentration of 25%, and EDTA 17% with green okra fruit extraxt concentration of 25%, which show a significance value of 1.000 ($\alpha>0.05$), meaning there is no significant difference.

DISCUSSION

Figure 1 shows differences in *S.mitis* colony growth on petri dishes. A total of 36 colonies were observed in the presence of the 6.25% extract, 12 colonies with the 12.5.% extract, and no colonies with the 25% extract. Similarly, no bacterial colonies were found in the 2.5% NaOCl and EDTA groups, whereas 159 colonies were observed in the aquadest (negative control) group. Based on the results, the number of bacterial colonies decreased as the concentration of okra fruit extract increased. This study aimed to determine the MBC of okra fruit extract against *S.mitis*. The MBC is defined as the lowest concentration that can kill 99.9% of the tested bacteria.[14] In this study, the MBC of the okra extract was identified at a concentration of 25%.

The MBC value observed in this study was higher than that reported by Lestari et al. (2024), who determined the MBC of okra fruit extract against *Enterococcus faecalis* to be 12.5%.[11] This difference may be attributed to variations in the extraction methods used in the two studies. In the present study, the okra was dried for 12 days, whereas Lestari et al. (2024) utilized oven-drying. Drying is a process aimed at removing moisture from a solid material to inhibit microbial growth, thereby preventing spoilage caused by chemical reactions and preserving the active compounds within the material. The drying process can lead to structural, chemical, and nutritional value.[18,19] This is supported by the findings of Febrinda *et al.* (2023), who

reported that air-drying resulted in the lowest flavonoid content in *Eleutherine bulbosa* (Mill.) and produced the smallest inhibition zone against *Staphylococcus aureus*.[18]

The antibacterial activity of green okra fruit extract is attributed to its bioactive coumpounds, including alkaloids (6.88%), flavonoids (5.01%), saponins (4.02%), tannins (3.81%), and terpenoids (3.22%), each of which exhibits antibacterial effect through different mechanism.[11] Alkaloids disrupt bacterial peptidoglycan components, preventing the formation of a complete cell wall and weakening its strength.[20] Flavonoids inhibit nucleic acid synthesis and inhibit DNA gyrase, which is an essential enzyme for bacterial replication.[21,22] Saponins exhibit antibacterial properties through saponification reactions or reactions involving triglycerides (NaOH) and alkali (KOH), which reduce cell wall surface tension and lead to cell wall lysis. The lyobipolar nature of saponins enables them to interact with plasma membranes, which in aqueous solutions, leads to a reduction in surface tension.[20,23]

Tannins affect bacterial growth through the inhibition of extracellular bacterial enzymes and eliminating of the substrates required for bacterial growth.[21,22] Terpenoids targeting the bacterial cell membrane, it has lipophilic characteristics that enable to easily penetrate bacterial cell wall, disrupting transmembrane proteins (porins) and hindering important cellular processes.[24] Porins act as gateways for compound entry and exit; their damage leads to bacterial nutrient deficiencies.[21]

The results of the Mann-Whitney test, shown in Table 2 revealed significant differences between green okra fruit extract concentrations of 6.25%, 12.5%, and aquadest compared to green okra fruit extract concentrations of 25%, NaOCl 2.5%, and EDTA 17%. This difference is attributed to the observed colony growth in the groups treated with green okra fruit extract concentrations of 6.25%, 12.5%, and aquadest, which showed 36, 12, and 159 colonies. In the research group green okra fruit extract concentrations of 25%, NaOCl 2.5%, and EDTA 17% showed no growth of *S.mitis* colonies, indicating that all three research groups have the same bactericidal properties against *S.mitis* bacteria, but green okra fruit extract requires a higher concentration.

NaOCl 2.5% and EDTA 17% are pure compounds with strong antibacterial properties. EDTA's antibacterial mechanism comes from its ability to remove Mg^{2+} and Ca^{2+} ions on the bacterial cell wall, disrupting biofilm stability and rendering bacteria more vulnerable to antibacterials.[25] NaOCl exhibits bactericidal activity against *S.mitis* through the action of active chlorine, which inhibits key enzymatic pathways, disrupts metabolic functions, and ultimately induces bacterial cell death.[5]

CONCLUSION

The concentrations of 6.25% and 12.5% green okra fruit extract (*Abelmoschus esculentus*) still showed growth of *S.mitis* colonies. The minimum bactericidal concentration (MBC) is found at a concentration of 25%. The implication of this study is that green okra fruit extract has the potential to be developed as an alternative root canal irrigant due to its demonstrated antibacterial activity. However, further investigation into the optimal drying method for okra simplicia is necessary to enhance the preservation of bioactive compounds responsible for its antibacterial effects. Subsequent studies are also recommended to evaluate its in vivo efficacy and toxicity to ensure both safety and clinical applicability.

Declaration by Authors

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