Efficacy of green tea leaf extract (*camellia sinensis*) with NaOCl 2.5% against *enterococcus faecalis* as an alternative solution for root canal irrigation

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Abstract

Objective: *Enterococcus faecalis* is a coccal gram-positive bacterium with facultative anaerobic feature. NaOCl have been proven effective against *enterococcus faecalis* for root canal irrigation process. Green tea leaf extract (*camellia sinensis*) can be chosen as an alternative solution for root canal irrigation. The purpose of this study was to determine the difference in efficacy between green tea leaf extract (*camellia sinensis*) and NaOCl 2.5% against the growth of *enterococcus faecalis* bacteria.

Material and Methods: This is a laboratory experimental study with "posttest only group" design. The first step of the trial was done by making green tea leaf extract and then determine the

lowest concentration on which the first solution became clear. The concentrations that were tested are 1.5%, 2.5%, 3.5%, 4.5% and 5.5%.

Results: The results was found that the minimal inhibition concentration of the green tea leaf extract was 1.5%. The anti-bacterial effect testing method was using diffusion method to differentiate inhibition zone of the green tea leaf extract solution of various concentrations that were being tested and compare it with NaOCl 2.5%.

Conclusion: Green tea leaf extract (*camellia sinensis*) is effective in inhibiting the growth of *Enterococcus faecalis* bacteria.

Keywords: *Enterococcus faecalis*, Green tea leaf extract, NaOCl 2.5%

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Introduction

Important stages in the infected root canals treatment are preparation, sterilization and filling. Root canal preparation supports the sterilization process that produces good outcome to obtain maximum results. Preparation stage is required in root canal irrigation materials to remove necrotic tissue, dentin debris pile and to prepare the root canals so that it will enable the preparation and reduce the number of microorganisms in the root canals.² Infections that occur in the root canals are caused by micro-organisms that gain entry into the periapical and pulp tissue. On occasions when endodontic treatment failed, *enterococcus faecalis* is one type of bacteria that was frequently found in the root canals. Clinical impact caused by virulence of these bacteria is an acute apical periodontitis, chronic periodontitis, apical periodontitis exacerbation, marginal periodontitis and periradicular abscess.⁴ An irrigation solution of root canals should be able to dissolve both organic and inorganic dirt, lubricated endodontic instruments, ability to kill microbes, intoxic effects and economical properties. The best irrigation solution has maximum antimicrobial ability with minimal toxicity. Irrigation materials which are commonly used

have antiseptic property which is a substance that can inhibit the growth of microorganisms in vitro and in vivo of living tissue. The effectiveness and toxicity of the irrigation solution highly depend on the concentration, temperature and time.³ Materials that can be used for irrigation for example are: hydrogen peroxide (H₂O₂), NaOCl, EDTA and chlorhexidine gluconate.¹ Sodium hypochlorite (NaOCl) solution is an irrigation solution frequently used. It is also used as antiseptic and inexpensive lubricants with concentration range from 0.5 to 5.25%. Free chlorine contained in NaOCl dissolves vital and necrotic tissues by breaking down proteins into amino acids. NaOCl has antibacterial properties but it is unable to clean whole of the smear layer. Decreasing levels of NaOCl solution can reduce the toxicity, antibacterial effects as well as its potency to dissolve the tissue. While the volume increase or heating NaOCl may escalate the effectiveness as a canal solution.⁵ Based on the preparation process, tea products can be divided into three types, consist of green tea, oolong tea and black tea. The types of tea that are widely consumed in the world are black tea 78%, green tea 20%, and oolong tea 2%. Black tea is widely consumed by the people of Europe, North America and North

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Africa (except Morocco). Whereas, green tea is widely consumed by the people of Asia, including Indonesia and oolong tea is widely consumed by the population of China and Taiwan.⁷ Indonesia has a massive tea plantations. Tea plants growing in Indonesia are dominated by assamica variety that originated from India. Tea plants that grow in Japan and China are Sinensis variety. Assamica variety of tea contains greater catechins compared to others. Tea has many benefits for the body because it contains polyphenols that have potential as an antioxidant that protects the body from free radicals. Antioxidant properties in tea are more powerful compared to antioxidants found in fruits and vegetables. Some of the tea benefits have been identified such as decreasing cholesterol level and the osteoporosis risk, serving as anti-virus, deodorizes, maintaining oral health, improving the cognitive and psychomotor condition of adults, preventing blood clot, coronary heart disease and liver disease, as well as the growth and development of cancer, particularly stomach, esophagus, and skin cancer.⁷ Alternative material that can be used as irrigation is herbs. One of the plants that contain antibacterial activity is green tea (*camellia sinensis*). The Section that contains antibacterial in green tea leaves is phenols or polyphenols substance (catechins, tannins and flavanols) and non-phenol substance (alkaloids and flour) which can inhibit and kill bacteria. From various researches of herbs, green tea is an alternate solution for root canal irrigation.⁸ Based on the facts explained the researcher would like to figure out the effectiveness of green tea leaf extract (*camellia sinensis*) compared to 2.5% NaOCl as an alternative root canal irrigation solution which will be examined against the bacteria *enterococcus faecalis*.

Material and Methods

Type of research that was performed in this case was experimental laboratory research, with "posttest only group design". This research was conducted at Microbiology Laboratory, Faculty of Medicine and Phytochemistry Laboratory, Faculty

of Pharmacy, Hasanuddin University, Makassar Indonesia. This study required 18 samples for the 3 groups. The subjects in this research were *enterococcus faecalis* bacteria. The extraction method used in this research was the maceration method. This study was firstly commence by determining the concentration of Minimum Inhibitory Concentration (MIC) then followed by measuring the inhibition zones formed in the agar medium.

Results

This study administered 18 samples divided into 3 petri dishes containing green tea leaf extract (*camellia sinensis*) with various concentrations compared to 2.5% NaOCl. After observing the petri dishes previously incubated for 24 hours at temperature of 37°C, the effectiveness of green tea leaf extract with NaOCl 2.5% against the growth of *enterococcus faecalis* was obtained. In the BHIB medium, after the leaf extract of green tea (*camellia sinensis*) was provided and incubated for 24 hours, it revealed that all of the concentrations did not undergo turbidity, from the lowest to highest concentrations. Based on these tests, we can conclude that the MIC of the extract of green tea leaves is at 1.5% concentration.

Table 1 it was conducted by measuring the inhibition zone formed on the surface of the bacterial culture media. It could be identified that the inhibition zone formed by 2.5% NaOCl was greater than that of green tea leaf extract. However, green tea leaf extract was also capable of inhibiting the growth of *enterococcus faecalis* bacteria. It was demonstrated by the existence of inhibition zone so that we could conduct measurement as shown above. Based on the research results, the width of inhibition zone at concentrations 1.5% was only 7.15 mm and continuously ran into increasing 5.5% concentration up to 9.25 mm. Meanwhile, the highest value of NaOCl inhibition zone area was about 12.75 mm. Green tea leaf extract at 1.5% concentration was the minimum inhibitory concentration which can inhibit the growth of *enterococcus faecalis* bacteria. We obtained data that green tea leaf extract was able to inhibit the growth of *enterococcus faecalis* bacteria. The higher the concentration of green tea leaves extract, the greater the inhibition zones that were found in paper disc.

Table 2 the result of one-way ANOVA test with 95% confidence level ($p < 0.05$) is to identify whether there is any difference or not in effectiveness between green tea leaf extract and 2.5% NaOCl in inhibiting *enterococcus faecalis* bacteria. However, from this study there is no significant difference

Table 1 Results of measurement of the inhibition zones (mm)

Petri Dish	The Extract of green tea					NaOCl 2.5%
	1.5%	2.5%	3.5%	4.5%	5.5%	
A	6.6	7.8	9.8	9.4	10.05	11.1
B	6.8	7.4	6.8	7.8	7.9	19.85
C	8.05	8.9	8.5	8.9	9.8	7.3
Total	7.15	8.03	8.36	8.7	9.25	12.75

Table 2 The difference of the inhibition zone average between green tea leaf extract and 2.5% NaOCl against enterococcus faecalis bacteria

Treatment	Mean±SD	p-Value
1.5%	7.15 ± 0.79	0.275
2.5%	8.03 ± 0.78	
3.5%	8.34 ± 1.50	
4.5%	8.70 ± 0.82	
5.5%	9.25 ± 1.18	
NaOCl 2.5%	12.75 ± 6.44	
Total	9.04 ± 2.98	

($p > 0.05$) and the probability value is 0.275. Because ANOVA test results are not significant, it cannot be continued to LSD analysis.

Discussion

Based on the research that has been conducted, the MIC test reveals that in the smallest concentration (1.5%) showed the clarity on the tube. And greater the concentration of green tea leaf extract, it appears more clear on the tube. It indicates that the green tea leaf extract with those concentrations can inhibit the growth of enterococcus faecalis bacteria in the root canals.

To examine the effectiveness of green tea leaf extract against enterococcus faecalis bacteria using diffusion method was used to observe the inhibition zone formed in the petri dish. This study administered triple replications in order to the results of measuring inhibition zone obtained which are more accurate. Measurement to determine the area of inhibition zone was performed by using a caliper to measure the diameter of clear zone, including the paper disc that had diameter about 6mm. Measurement was performed vertically, horizontally, and diagonally and then the scores were summed and divided into three to obtain the average value of the inhibition zone.

The results obtained from the tests of all green tea leaf extract concentration showed that there was inhibition zone. On examining the effectiveness of 2.5% NaOCl, the inhibition zone was also formed although it was larger than that of the green tea leaf extract. The size of the inhibition zone formed by using 2.5% NaOCl after averaging was 12.75 mm. where as the green tea leaf extract at 1.5% concentration had 7.15 mm, at concentration 2.5% had 8.03 mm, at 3.5% concentration had 8.36 mm, at concentration 4.5% has 8.7 mm and 5.5% concentration had 9.25 mm inhibition zone. The inhibition zones after incubation period for 24 hours indicated that the bacteria residing in the area could not grow due to the influence of the test materials such as green tea leaf extract and 2.5% NaOCl which diffused out

of paper disc into the surrounding area. The enterococcus faecalis bacteria are facultative anaerobic gram-positive bacteria with cocci shape which have thick peptidoglycan cell wall, but if there is damage or obstacle in their formations, the cells would be death. One of the materials that have antibacterial activity is green tea leaf (camellia sinensis).³³ The ability of green tea leaf extract (camellia sinensis) in inhibiting the growth of enterococcus faecalis bacteria is caused by the chemical substances contained in green tea leaf which is known to have antibacterial power. The highest chemical substance in the green tea leaves is polyphenols or catechins. High polyphenol content in green tea can be administered to kill bacteria as well as bacteria that cause disease in the oral cavity including periodontal disease. Catechins contained in green tea leaf can be either bacterio-static or bactericidal that depend on their concentration. The catechin content in green tea also has antimicrobial power against streptococcus mutans. Catechins work by denaturing the proteins from bacteria.²²

Denaturated proteins will lose physiological activities and unable to function properly. The changes of the protein structure in the bacteria cell wall will increase the cell permeability so that the cell growth is inhibited and then the cell becomes damaged. Because of the protein cell denaturation, all the cell metabolic activities are catalyzed by the enzyme so that the bacteria cannot survive.³⁴

Based on this study, it proves that the greater the concentration of green tea leaf extract, the greater the antibacterial activity occurs. The higher concentration of antibacterial agent provided, the stronger the occurrence of antibacterial activity. We can assume that the diameter of the inhibition zone is directly proportional to the concentration level.³⁵

In several in vitro studies conducted against NaOCl antibacterial activity reveal that this solution is the best solution and it is used frequently in root canal treatment. Based on the research conducted by Gomes, regarding the effectiveness of NaOCl at concentrations (0.5%, 1%, 2.5%, 4% and 5.25%) compared with chlorhexidine gluconate at concentrations (0.2%, 1% and 2%) are that both of the irrigation solutions are effectively the same in inhibiting the growth of enterococcus faecalis bacteria but it is at different time. Meanwhile, according to Berber, the most effective concentration of NaOCl in inhibiting enterococcus faecalis is 5.25% then followed by 2.5% concentration.³⁶

The green tea leaf extract is able to inhibit the growth of Enterococcus faecalis bacteria within 6 minutes. It is due to the content of catechins found

in green tea leaves that have high antioxidant and antibacterial. The catechin content that has antibacterial power can kill pathogenic microorganisms such as *E. coli*, streptococcus mutans, dysenteriae shigella and vibrio cholerae.³⁷

Green tea leaf extract with 0.5%–3% and 3.5%–6% concentrations indicate that the green tea leaf extract at a concentration of 1%–1.5% has 9 mm, then at 2.5% concentration has 20 mm and at 3% concentration has 30 mm of inhibition zone. Moreover from the results, green tea leaf extract at 3.5%–6% concentrations reveals no bacterial growth. Moreover green tea leaf extract at 3.5% concentration has antibacterial activity in inhibiting the growth of enterococcus faecalis.³⁷

Green tea leaf extract (*camellia sinensis*) and 2.5% NaOCl have the same effectiveness in inhibiting the growth of enterococcus faecalis bacteria. Even in the smallest amount of concentration 1.5%, the extract of green tea leaves can inhibit the growth of enterococcus faecalis bacteria. It is demonstrated by forming the inhibition zone. Meanwhile, higher the concentration of green tea leaf extract is provided, the greater the inhibition zone is formed. Therefore we can conclude that green tea leaf extract can inhibit the growth of enterococcus faecalis bacteria, but NaOCl 2.5% has better activity.

There are differences in antibacterial effects between the extracts of green tea leaves (*camellia sinensis*) with 2.5% NaOCl against enterococcus faecalis as alternative root canal irrigation solutions. The higher the concentration of green tea leaf extract (*camellia sinensis*), the greater inhibition zone is formed against the enterococcus faecalis bacteria. The result of minimal inhibitory concentrations of green tea leaf extract at 1.5% concentration was 7.15 mm whereas at 2.5% of NaOCl is 12.75 mm of inhibition zone.

Conclusion

Green tea leaf extract (*camellia sinensis*) is effective in inhibiting the growth of enterococcus faecalis bacteria. However, 2.5% NaOCl is better in inhibiting the growth of enterococcus faecalis bacteria. It is because the inhibition zone formed by 2.5% NaOCl is much larger than that of green tea leaf extract (*camellia sinensis*).

Conflict of Interest

The authors report no conflict of interest.

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