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Antibacterial Assay of Green Tea (*Camellia Sinensis L*) Against the Growth of *Enterococcus faecalis*

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ABSTRACT
Background: *Enterococcus faecalis* (*E. faecalis*) is often reported as an infectious agent in tooth root canals. Green tea leaves (*Camellia sinesis L*) contain active substances, namely catechins, which can act as an antibacterial. Research. **Objective:** To determine the antibacterial effectiveness of green tea extract (Camellia sinensis l) against the growth of Enterococcus faecalis bacteria. **Methods:** Researchers carry out tests in the laboratory, which are called laboratory experimental tests. The form of this research is Post-test Only Control Design. Researchers took samples using a purposive sampling technique with four treatments and six repetitions. The statistical test used is the Kruskal-Wallis test. **Results:** Based on the results of Kruskal Wallis, the p-value shows a value of 0.011 or a p-value of less than 0.05, which indicates that there is a significant difference between the Camellia sinensis L 25%, 50%, 75% treatment, and the positive control. **Conclusion:** Green tea extract (Camellia sinensis l) with concentrations of 25%, 50%, and 75% can inhibit *E. faecalis*' growth.
Keywords: Root canal irrigation, green tea (*Camellia sinensis L*), *Enterococcus faecalis*

ABSTRAK
Latar Belakang: *Enterococcus faecalis* (*E. faecalis*) sering dilaporkan sebagai agen infeksi dalam saluran akar gigi. Daun teh hijau (camellia sinesis, L) mengandung zat aktif yaitu katekin yang dapat beran sebagai antibakteri. **Tujuan:** Untuk mengetahui bagaimana efektivitas antibakteri ekstrak teh hijau (*Camellia sinensis L*) terhadap pertumbuhan bakteri Enterococcus faecalis. **Metode :** Peneliti melakukan pengujian di laboratorium atau disebut dengan uji eksperimen laboratorium. Bentuk penelitian ini adalah Post test Only Control Design. Peneliti mengambil sampel menggunakan teknik purposive sampling dengan 4 perlakuan dan 6 kali pengulangan. Uji statistik yang digunakan adalah uji Kruskal Wallis. **Hasil:** Berdasarkan hasilKruskal Wallis Nilai p-value menunjukkan nilai 0,011 atau p-value kurang dari 0,05 yang menunjukkan bahwa terdapat perbedaan yang signifikan antara perlakuan Camellia sinensis L 25%, 50%, 75% dengan kontrol positif. **Kesimpulan:** Ekstrak teh hijau (Camellia sinensis l) dengan konsentrasi 25%, 50% dan 75% mampu menghambat pertumbuhan Enterococcus faecalis.
Kata Kunci : Irigasi saluran akar, teh hijau (*Camellia sinensis L*), *Enterococcus faecalis*

1. Introduction

In Indonesia, dental and oral disease is the most common complaint. Examples include caries or cavities, which will later progress to pulp disease, which then becomes periapicaldisease.[1]Pulpal and periapical diseases can be treated with endodontic treatment.[2] Endodontic treatment is a procedure performed in dentistry. This is done so that theteeth that have damaged the pulp can be maintained so they don't need to be removed.[3,4] Microorganisms appearing after root canal filling are one of the main reasons root canal treatment fails. The *E.faecalis*, *Streptococcus anginosus*, *Bacteroides gracilis*, and *Fusobacterium nucleatum* are often found in infected root canals.However, the most common bacteria found in cases after root canal treatment are *E. faecalis*. Approximately 50% of *E. faecalis* is in infected root canals in failed root canal treatment cases. This microorganism has a gram-positive and facultative anaerobic coccus form.[3,5,6]

Chlorhexidine gluconate (CHX) is an irrigation solution used for approximately 60 years and used in various medicines and other medical devices. CHX has been used as a general-purpose antiseptic in humans. 5.25% NaOCL and 2% CHX have more or less the same antibacterial potential. CHX can adhere to root canal walls. Therefore, CHX provides a long-lasting and broad-spectrum

antibacterial effect.[7,8] *Chlorhexidine gluconate* (CHX) with a concentration of 0.2% is used as an irrigant because of its broad antimicrobial activity, substantivity, and low cytotoxicity.CHX is effective in inhibiting Enterococcus faecalis bacteria and has also been used as a root canal disinfectant. In addition, CHX is water soluble and has a low level of toxicity. On theother hand, organic matter and necrotic tissue found in the root canal system are not soluble by CHX. [5,9,10]

Irrigation materials derived from nature can be a choice of root canal irrigation materials because they have bacteriostatic and bactericidal effects. Besides being more affordable and accessible than chemical drugs, natural ingredients are also consideredto have fewer side effects. The human body is far more sensitive to drugs made from plantcomponents than chemical drugs, which is why natural medicinal plants exist.[11,12] Malino is a tea-producing region in South Sulawesi with a temperature range of 24oC, making tea plants thrive there.[13,14] Green tea leaf contain active substances called catechins. The properties of catechins in green tea are antimicrobial because they contain pyrogallol groups and gall oil groups. The tertiary structure of compounds containing catechol or pyrogallol groups and their gall oil groups controls the inhibitory effect of the poison.

The antibacterial activity in green tea has been proven in various studies conducted by experts worldwide. One of the results is that green tea can inhibit the activity of Salmonella typhi and Escherichia Coli (bacteria that cause typhus and diarrhea). The benefits of green tea for health are that it can prevent various cancers, maintain heart health, prevent kidney disease, prevent dental caries, and prevent oxidation.[15,16] Based on a study by Wijaya S, et al. (2021) on the topic of research on the antibacterial effectiveness of green tea extract against Streptococcus mutans bacteria, Green tea concentrations used are 3.125%, 6.25%, 12.5%, 25% and 50% with one Dimethyl Sulfoxide (DMSO) group as a negative control. Green tea in Wijaya's research produced the following inhibition zones: 1) strong at 21% and 50%; 2) moderate at concentrations of 3.125%, 6.25%, and 12.5%; 3) weak in the DMSO group.

Green tea extract has antibacterial activity against the growth of *Streptococcus mutans*. This is evident from the diameter of the inhibition formed from green tea leaf extract. Green tea leaf extract with a concentration of 50% effectively inhibits the growth of S. mutans bacteria.[17] Asmardi A et al. (2014) explain a theory that the amount of inhibition follows the high concentration of a material. Thus, it can be stated that the diameter of the inhibition zone and the concentration level are directly proportional.[18] Based on this, researchers are interested in examining whether green tea extract at concentrations of 25%, 50%, and 75% can inhibit the growth of Enterococcus Faecalis bacteria.

2. Material and Methods

This research design is laboratory experimental (true experimental), a test carried out in a laboratory with a post-test-only control design to measure the effect of treatment on the experimental group by comparing the group with the control group. This study used four treatment groups of green tea leaf extract with concentrations of 25%, 50%, 75%, and chlorhexidine (CHX) as positive controls. Each green tea and CHX treatment in this study was repeated five times.

2.1. Green tea extract (Camellia Sinensis L)

Green tea leaves picked from Malino are taken to the laboratory and washed thoroughly under running water, then green tea leaves are cut to small size and dried using a drying cabinet with a temperature of 50 ^{o}C for 1-2 days. Dried green tea leaves were put in a maceration container, then covered with 96% ethanol solvent, left for 1x24 hours at room temperature, protected from light while occasionally stirring, and filtered using filter paper. Next, the leaf filter green tea result is extracted using a rotary evaporator with a temperature of 50 ^{o}C for 4. The clock helps separate solvents with green tea extract to obtain this extract.

2.2. Preparation Enterococcus faecalis

Begin by stirring 3.4 grams of Mueller Hinton Agar in 100 mL of distilled water (aquades) until the agar completely dissolves. Next, transfer the MHA solution into an appropriate container that can withstand high temperatures and sterilize it in an autoclave at 121°C for 25 minutes to eliminate any contaminating microorganisms. After sterilization, and once the MHA has slightly cooled but is still liquid, carefully pour the medium into sterile petri dishes, ensuring the environment is clean and free from contaminants. Once the MHA is in the Petri dishes, divide the bottom of each dish according to the treatment groups to determine the regional limit of each

treatment on the MHA, allowing for multiple antibiotics or conditions to be tested on the same plate. Prepare the culture by taking Enterococcus faecalis from its stock using a sterile inoculating loop (ose), as this bacterium is frequently used in antibiotic susceptibility tests. Gently streak the E. faecalis onto the surface of the Mueller Hinton Agar in the petri dishes, ensuring even distribution and avoiding digging into the agar surface. Incubate the inoculated petri dishes at 35-37°C for 18-24 hours, allowing the bacteria to grow and form visible colonies. After the incubation period, observe the growth patterns of E. faecalis. If performing an antibiotic susceptibility test, measure and compare the zones of inhibition (areas where bacteria did not grow around antibiotic disks) against standard tables to determine the sensitivity or resistance of the bacteria to the antibiotics tested.

2.3. *Antibacterial Assay*

After the Mueller Hinton Agar medium solidifies, a 6 mm diameter paper disk is embedded with varying concentrations of green tea extract, specifically 25%, 50%, and 75%, along with a positive control of 0.2% Chlorhexidine gluconate, and then placed on the surface of the MHA corresponding to the predetermined quadrants. Once the disks are in place, the petri dish is closed and incubated for 24 hours at 37°C. Following the incubation, an observation of the inhibition zones is carried out. These zones are characterized as clear, uncolonized areas surrounding the paper disks on the bacterial medium and are indicative of the antibacterial activity of the substances. The size of the inhibition zones is measured using a caliper, recorded in millimeters (mm), to determine the effectiveness of each concentration of green tea extract and Chlorhexidine gluconate against bacterial growth.

2.4. *Data Analysis*

The antibacterial effect of *Enterococcus faecalis* was analyzed with Kruskall Wallis and Post Hoc tests, and the significance was p<0,05.

3. Result and Discussion

Table 1, as described, indicates the results from the Kruskal-Wallis test comparing the antibacterial effects of Chlorhexidine gluconate (CHX) and various concentrations of green tea extract treatments on Enterococcus faecalis. The data shows that the lowest average inhibition zone size in the CHX group was 14.71 mm, while the highest average inhibition zone size was 22.80 mm for the 75% concentration of green tea extract treatment. The Kruskal-Wallis test yielded a p-value of 0.011, less than the significance level of 0.05. This result indicates statistically significant differences in the inhibitory effects among the different treatment groups.

Given that the Kruskal-Wallis test has established overall significance among the groups, the next step involves pinpointing the specific treatments that differ significantly. This is where the Mann-Whitney test comes into play as a Post Hoc analysis. The Mann-Whitney test compares differences between two independent groups when the dependent variable is ordinal or continuous but not normally distributed. In this case, it's applied pairwise to each treatment group against the others to find out which specific pairs of treatments (e.g., CHX vs. 25% green tea extract, CHX vs. 50% green tea extract, etc.) show significant differences in the inhibition of Enterococcus faecalis growth. By conducting the Mann-Whitney tests, researchers can conclude that there is a significant difference in antibacterial activity among the treatments and identify which treatments are most effective or significantly different from each other. This detailed information is crucial for understanding the effectiveness of each antibacterial agent and guiding future treatment or research directions.

Table 1. Results of *Kruskall Wallis* Test

Camellia Sinensis L	N	Mean	Std. Deviation	p-value*
CHX	6	14.71	1.28	0.011*
25%	6	16.74	2.43	
50%	6	19.98	3.98	
75%	6	22.80	4.24	

* Uji *Kruskall Wallis*, *signifikan (p<0,05)

Table 2 presents the results of a pairwise comparison between the Chlorhexidine gluconate (CHX) group and the 75% green tea extract treatment group using the Mann-Whitney test, a non-parametric test used for comparing differences between two independent groups. The p-value obtained from this comparison is 0.025, less than the significance level of 0.05. A p-value of 0.025 indicates a statistically significant difference between the CHX group and the 75% green tea extract

treatment group in their ability to inhibit the growth of Enterococcus faecalis. Specifically, because the p-value is below the 0.05 threshold, we can reject the null hypothesis that there is no difference between the two groups' median inhibition zones and conclude that there is a statistically significant difference in the antibacterial effectiveness of the two treatments. This significant result, particularly in the comparison between the CHX group and the 75% green tea extract group, suggests that the 75% green tea extract treatment has a notable effect on inhibiting the growth of E. faecalis compared to the CHX group. These findings support the consideration of high-concentration green tea extract as a potentially effective antibacterial agent against Enterococcus faecalis and highlight the importance of exploring alternative or complementary treatments to traditional antimicrobial agents like CHX.

Table 2. *Post Hoc Multiple Comparisons* values

Camellia Sinensis L	Mean	Post Hoc			
		CHX	25%	50%	75%
CHX	14,71	-	0.100	0.054	0.025*
25%	16,74		-	0.045*	0.037*
50%	19,98			-	0.037*
75%	22,80				-

*signifikan (p<0,05)

The comparison results between the 25% concentration of green tea extract treatment with an average of 16.74 and the 50% concentration with an average of 19.98 obtained an average difference in inhibitance of 3.24. The test result obtained a p-value of 0.045, less than 0.05 (p-value<0.05). This shows a significant difference between the 25% and 50% treatment groups against inhibitivity. These results showed that the 50% concentration of green tea extract treatment group was significantly better than the 25% group because it inhibited bacterial growth. Meanwhile, comparing the 25% concentration of green tea extract treatment with an average of 16.74 and the 75% concentration treatment with an average of 22.80, an average difference in inhibitivity of 6.06 was obtained. The test result obtained a p-value of 0.037, less than 0.05 (p-value<0.05). This shows a significant difference between the 25% and 75% treatment groups against inhibitivity. These results showed that the 75% concentration treatment group was significantly better than the 25% group because it inhibited bacterial growth.

The comparison result between the 50% concentration of green tea extract treatment with an average of 19.98 and the 75% concentration treatment with an average of 2.82 obtained an average difference in inhibitivity of 2.82. The test result obtained a p-value of 0.037, less than 0.05 (p-value<0.05). This shows a significant difference between the 50% and 75% treatment groups against inhibitivity. These results showed that the 75% concentration treatment group was significantly better than the 50% group because it inhibited bacterial growth. Based on the results of the overall comparison of the green tea extract treatment group of 25%, 50%, and 75% of green tea in the growth of *E. faecalis*, it was shown that the treatment with 75% extract was most influential in inhibiting the growth of *E. faecalis*.

The research that is different from the results of this study is from Endarini L.H. (2021), explaining that the test using green tea leaf extract with concentrations of 25%, 50%, 75%, and 100% shows that the barrier zone at high concentrations of the resulting inhibitivity is smaller. This is influenced by the bacterial wall, which consists of lipoproteins containing protein molecules, namely porins and oligosaccharides. The hydrophobic properties of the extract are contrary to the hydrophilic properties of porin, so protein molecules in the extract components have more difficulty entering the bacteria. As a result, this can affect the working activity of green tea ethanol extract in inhibiting the growth of Escherichia coli.[20]

In this study, the results of the inhibited zone were obtained with a concentration of green tea extract of 25%, 50%, and 75% in units (millimeters), namely 16.74 mm, 19.98 mm, and 22. The results obtained from 25% concentration, 50% showed that being at 11-20 mm means that it is included in the strong category, and 75% concentration shows that it is at 21-30 mm means that it is included in the very strong category. The average diameter of the inhibitory zone produced by Chlorhexidine gluconate (CHX) as a positive control of 14.71 mm shows the diameter of the inhibitory zone is smaller than the diameter of the barrier zone produced by green tea extract (Camellia sinensis L). This is in line with research conducted by Werdiningsih M. et al. (2015), explaining that in the results of his research using 100% raisin infusion is assumed to be an effective concentration when compared to 0.2% chlorhexidine because it has a more significant inhibitory zone than other concentrations of raisin infuse groups.[21]

Based on the results of this study, green tea extract can inhibit the growth of Enterococcus faecalis bacteria. This is influenced because of the content of polyphenols (flavonoids and tannins) that can inhibit bacteria. This aligns with Bayi B. et al. (2022) research, explaining that flavonoids are polyphenolic compounds that have effects such as antioxidants, antitumors, anti-inflammatory, antibacterial, and antiviral. Flavonoids work by inhibiting nucleic acid synthesis by attaching to the GyrB DNA gyrase subunit and inhibiting the activity of the ATPase enzyme, which prevents bacterial DNA replication. Catechins inhibit bacteria by damaging the cytoplasmic membrane of bacteria, which limits the entry of nutrients needed by bacteria to produce energy. Therefore, bacteria will be hampered in their growth and experience death.[22,23]

This research is in line with research conducted by Annita & Panus H (2018) with the title "Green Tea Leaf Extract Inhibiting Power (Camellia sinensis l) Against Streptococcus Mutans Bacteria" concluded that green tea leaf extract can inhibit the growth of S.mutans bacteria. At 10% concentration, the average drag zone is 14.81 mm; 20% concentration is 18.86 mm; 30% concentration is 20.91 mm; 40% concentration is 20.22 mm; 50% concentration is 21.53 mm; and at 100% concentration is 26.09 mm.[24] Based on the explanation above, it can be concluded that green tea leaf extract can inhibit the growth of *E. faecalis*, so that it can be used as a root canal irrigation material.

4. Conclusion

Green tea extract *(Camellia Sinensis L)* effectively inhibits the growth of *E. faecalis*. At a concentration of green tea extract *(Camellia Sinensis L),* 75% has the largest inhibitory zone in inhibiting the growth of *Enterococcus faecalis* with an average barrier zone diameter of 22.80 and is included in the very strong criteria.

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Authors Contribution

Contribution	Asmah N	Mattulada IK	Dodo AZA.
Concepts or ideas	√	√	√
Design	√	√	√
Definition of intellectual content	√		
Literature search	√	√	√
Experimental studies	√		√
Data acquisition	√	√	√
Data analysis	√	√	√
Statistical analysis	√		√
Manuscript preparation	√		√
Manuscript editing	√	√	√
Manuscript review	√	√	√

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