

Effectiveness of *Camellia Sinensis* L Extract in Inhibiting the Growth of *Staphylococcus aureus*

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ABSTRACT

Background: *Gingivitis* and periodontitis are the two most common periodontal conditions. *Staphylococcus aureus* was reported as a trigger. Compounds active from the green (*Camellia Sinensis* L) have been reported to be beneficial as antibacterial. **Objective:** know the effectiveness of extract tea leaves green 8%, 10%, and 12% in inhibiting bacterial growth of *Staphylococcus aureus*. **Method:** The study was conducted in a way that involved 25 power resistors. *S. aureus* was assessed with a diffusion disc technique with a post-test type-only control design. As well as test statistics *Kruskal-Wallis*. **Results:** Based on The results of the *Kruskal-Wallis* test showed a *p-value* of $0.000 < 0.05$. So, there was a significant difference between the 8%, 10%, and 12% extract treatments and the control group. **Conclusion:** Extracting leaves with a green concentration of 12% effectively hinders bacteria *Staphylococcus aureus*.

Keywords: Extract leaf Tea green, *Staphylococcus aureus*, gingivitis

ABSTRAK

Latar belakang: *Gingivitis*, dan periodontitis adalah dua kondisi periodontal yang paling umum terjadi. *Staphylococcus aureus* dilaporkan sebagai pemicu. Senyawa aktif dari the hijau (*Camellia Sinensis* L) telah dilaporkan berkasiat sebagai antibakteri. **Tujuan penelitian:** mengetahui efektivitas ekstrak daun teh hijau 8%, 10% dan 12% dalam menghambat pertumbuhan bakteri *Staphylococcus aureus*. **Bahan dan Metode:** Penelitian secara invtro dengan 25 daya hambat *S. aureus* dinilai dengan teknik *disk difussion* dengan tipe *post test only control* design. Serta uji statistik *Kruskal-Wallis*. **Hasil:** Berdasarkan hasil uji *Kruskal-Wallis* didapatkan hasil *p-value* $0,000 < 0,05$. Maka ada perbedaan yang signifikan antara perlakuan ekstrak 8%, 10% dan 12% dengan kelompok kontrol. **Kesimpulan:** Ekstrak daun the hijau konsentrasi 12% efektif menghambat bakteri *Staphylococcus aureus*.

Kata kunci: Ekstrak daun teh hijau, *Staphylococcus aureus*, gingivitis

1. Introduction

Gingivitis can generally affect teeth and mouth health. Microflora can potentially influence the health of other organs, such as teeth and mouth, often as the most important thing.^{1,2} Based on 2018 Riskesdas data, 57.6% of Indonesia's population experienced problems with healthy teeth and mouths, with a frequency of 67.8% in adults—periodontal diseases such as gingivitis and periodontitis. Gingivitis is a disease commonly experienced by people almost all over the world.^{3,4}

Gingivitis is an inflammatory reaction of the gingiva caused by biofilm accumulation in plaque along the gingival margin and an inflammatory response to bacteria. Causing changes in color, shape, consistency, texture, and bleeding of the gingiva are the main clinical signs of *gingivitis*.³ ^Abacteria called *Staphylococcus aureus* can cause gingivitis and is a bacteria that often causes infections in humans. One member of the Micrococcaceae family is *Staphylococcus*.^{5,6,7,8}

Natural ingredients are reported to contain several active ingredients that have the potential to be antibacterial. Tea green is a Medicinal plant often used in traditional medicine because it contains many active compounds, namely flavonoids, tannins, saponins, and potential alkaloids as antibacterial.^{9,10} Tea green is produced without a burning process (*enzymatic oxidation*), which is made by boiling fresh tea leaves to deactivate the phelonnase enzyme, which prevents the oxidation of catechins (antioxidants).^(11,12,13,14)

Based on research by Annita and Panus 2018, it is stated that green tea extract can hinder bacterial growth of *Staphylococcus aureus* with concentrations of 10%, 20%, 30%, 40%, And 50%.⁽¹⁵⁾ From the description, the researcher is interested in researching green tea leaf extract in inhibiting the growth of *Staphylococcus aureus* bacteria by using green tea leaf extract with concentrations of 8%, 10%, and 12%, and it is hoped that it can help reduce gingivitis.

2. Material and Methods

This research design is laboratory experimental (true experimental), namely a test carried out in a laboratory with a post-only control design, measuring the effect of treatment on the experimental group by comparing the group with the control group. This study used five treatment groups, namely green tea leaf extract with concentrations of 8%, 10%, and 12%, and CHX as a positive control and DMSO as a negative control. Each green tea treatment with CHX positive control and DMSO negative control in this study was repeated five times.

2.1. Extract Preparation

Tea leaves 1 kg of green tea obtained from the Malino tea garden, then stored for approximately 24 hours. After that, it is washed with running water until clean, and the green tea leaves are cut into small sizes and dried using a drying cabinet at a temperature of 50 °C for 1-2 days. Green tea leaves are considered dry if they are easy to break. The dried sample was weighed 200 grams, put in a maceration container, then 96% ethanol solvent was added, covered, and left for 1x24 hours at room temperature, protected from light, stirring occasionally, then filtered using filter paper. Next, the filtered green tea leaves are extracted using a rotary evaporator at a temperature of 50 °C for 4 hours, which helps separate the solvent from the green tea extract to obtain a thick extract. The objective of dilution is to get a specific concentration of green tea leaf extract. They are utilizing DMSO at a concentration of 15 ml per volume.

2.2. Preparation Culture Media

As much as 3.4 grams of Mueller Hinton Agar (MHA) was dissolved in 100 ml of distilled water using an Erlenmeyer tube covered with gauze and wrapped in paper. The media was sterilized in an autoclave at 121 °C for 25 minutes. The bottom of the petri dish was divided according to the number of paper disks that would be given to determine the area boundaries for each treatment in MHA. Next, use a syringe to insert 5 ml of medium into a sterile vial. Put one dose of bacteria in a vial containing the medium and then homogenize it. MHA was poured into a petri dish and left until solidified.

2.3. Inhibition Assay

After the medium has solidified, take a 6 mm paper disk using sterile tweezers, dip it in green tea leaf extract at concentrations of 8%, 10% and 12%, then put it in a petri dish. Then, dip a paper disk in CHX 0.2% positive control and DMSO as a negative control, and then put it in a different petri dish. Close the petri dish and wrap it using aluminum foil. Next, incubate it in an incubator for 1x24 hours at a temperature of 37 °C, and after that, observe the inhibition zone that forms.

2.4. Data Analysis

The antibacterial effect of *S. aureus* was analyzed with Kruskal Wallis and Post Hoc tests, and the significance was $p < 0.05$.

3. Result and Discussion

The normality test results showed that the *p-value* in each control group and the attitude in the negative control group for DMSO and positive for CHX 0.2% obtained a *p-value* of 0.604 mm and 0.724 mm > 0.05 ($p > 0.05$). The DMSO negative and 0.2% CHX positive controls were usually distributed. In the treatment group with 8% and 10% green tea leaf extract, *p values* were obtained at 0.095 mm and 0.748 mm > 0.05 ($p > 0.05$). The 8% and 10% concentration treatments have a normal distribution. Meanwhile, in the 12% concentration, a *p-value* of 0.006 mm was obtained, which was smaller than 0.05 ($p > 0.05$). This matter signifies that treatment Green tea leaf extract with a concentration of 12% is not normally distributed. The homogeneity test obtained a *p-value* of 0.004 mm < 0.05 mm. The Kruskal-Wallis test results in a *p-value* of 0.005, which is less than the commonly

used significance level of 0.05 ($p < 0.05$), it indicates that there are statistically significant differences among the groups being compared in terms of their median values (Table 2).

Table 1. Descriptive analysis of the effectiveness of tea extract on the inhibition of *Staphylococcus aureus*

Green tea extract	N	Mean	Std. Deviation	Data distribution	Homogeneity test
DMSO	5	8.32	1.01	0.604	0.004
CHX 0.2%	5	16.68	0.68	0.724	
8%	5	15.86	2.04	0.095	
10%	5	16.28	1.39	0.748	
12%	5	25.26	15.76	0.006	

In the context of your study, these groups are likely the different concentrations of treatments and the control (possibly CHX or another substance) used to inhibit the growth of *S. aureus*. A p -value of 0.005 is substantially low, suggesting that at least one of the treatment groups significantly differs in its inhibitory effect on bacterial growth compared to the others. Given this result, it's clear that the treatments and controls you're studying significantly inhibit bacterial growth.

Table 2. Effect of green tea extract on the inhibitory power of *Staphylococcus aureus* bacteria

Green tea extract	N	Mean	Std. Deviation	p -value
DMSO (K+)	5	8.32	1.01	0.005*
CHX 0.2% (K-)	5	16.68	0.68	
8%	5	15.86	0.68	
10%	5	16.28	2.04	
12%	5	25.26	1.39	

Based on the research findings, Table 1 shows that an inhibition zone with an average diameter of 15.68 mm appeared on the *blanc disk* at a green tea leaf extract concentration of 8%. The white disk yielded a concentration of 10% with an average diameter of the inhibitory zone measuring 16.28 mm, and 12% with an average diameter of the inhibitory zone measuring 25.26 mm. Each displayed an inhibitory zone that was relatively larger in diameter than the DMSO negative control, having an average inhibitory zone diameter of 8.32 mm but having an inhibitory diameter that was relatively no larger than the inhibitory zone created by the 0.2% CHX positive control, with an average diameter of the inhibition zone of 16.68 mm formed on the *blanc disk*. These results indicate that the positive control has a more significant inhibition zone than the groups with green tea leaf extract concentrations of 8%, 10%, and 12%. It is necessary to measure the area of the clear zone generated around the blank disk, as indicated by research conducted by Soraya and Fenny.¹⁶

This research does not agree with Oktarini and Amelia's research on the antibacterial activity of green tea leaf extract against *Staphylococcus aureus* and *Escherichia coli* bacteria, which found that the extract had an average inhibitory zone diameter of 6.8 mm and an average inhibitory power of 40%. There is no zone of inhibition for concentrations of 80% and 100% for all variations in extract concentration, with an average inhibitory power of 7.6 mm, an average 60% inhibitory power of 7.4 mm, and so on. This shows that *Escherichia coli* is not affected by green tea extract, while *Staphylococcus aureus* is affected. Enderini LH's research shows that the inhibition zone formed at high doses is smaller. This is influenced by the bacterial wall, which also consists of lipoproteins containing porin protein molecules and oligosaccharides as components of protein molecules. These porins are either hydrophilic or hydrophobic, and because of these differences in characteristics, protein molecules can enter the bacteria more slowly than extract components. As a result, this inhibits the extract's ability to prevent germs from multiplying. Likewise, according to Kumar's research, the active ingredients in green tea can stop the growth of bacteria. Especially green tea's high catechin and polyphenol content, which can damage bacterial cell membranes.^{17,18,19}

Polyphenol molecules known as flavonoids have anti-inflammatory, anti-tumor, antibacterial, and antiviral properties. Hydrogen bonding is the method by which flavonoids form protein complexes. Polyphenol chemicals bind to the H atoms in bacterial proteins to denature proteins in bacteria. Damage to the bacterial cell wall produces protoplasm, bacteria without walls. Damage to the bacterial cell wall will damage the cell membrane, particularly loss of cell membrane permeability, preventing the entry and exit of selective elements, including water, nutrients, and enzymes. Apart from flavonoids, tannins and saponins also have antibacterial properties. Saponins can damage membrane permeability and effectively reduce the surface tension of bacterial cell walls.

Furthermore, tannins have antibacterial properties. They can precipitate proteins and damage cell membrane defenses, which prevents bacterial growth. Tannins have antibacterial properties because they can precipitate proteins and damage cell membrane defenses, which prevents bacterial growth.^{20,21}

This research is in line with Wijaya's research on the antibacterial activity of green tea leaf extract against *Streptococcus mutans*, which produced an inhibitory power value of 5.18 mm at a concentration of 3.12%, 7.73 mm at a concentration of 6.25%, 9.23 mm at a concentration of 12.5%, 12.85 mm at a concentration of 25%, and 14.66 mm at a concentration of 50%. This shows that the antibacterial power of green tea leaf extract against *Streptococcus mutans* varies depending on the concentration. Parwata and Dewi's research also shows that the concentration of an antibiotic chemical impacts its effectiveness. The capacity of an antibacterial agent to inhibit bacteria increases with increasing concentration due to an increase in the amount of active antibacterial components.^{22,23}

4. Conclusion

The *Camellia Sinensis* L is effective in inhibiting the growth of *Staphylococcus aureus*. At a concentration of 12%, it has the largest inhibitory zone in inhibiting the growth of *Staphylococcus aureus*, with an average inhibitory zone diameter of 25.26 mm. It is included in the robust criteria based on the 8%, 10%, and 12 % comparison results.

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Authors Contribution

Contribution	Asmah N	Ilmianti	Abdi MJ	Irawati E	Nursaid H
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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