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Antibacterial Test of Cherry Leaves Ethanol Extract (*Muntingia calabura* L.) Against *Streptococcus mutans*

Annora Rizky Amalia[1]*, Riyan Setiyanto[1], Alfina Nurrahman[2], Ariska Rahmawati Utami[1], Dewi Weni Sari[1]

[1]Diploma of Pharmacy, Politeknik Indonusa, Surakarta, Indonesia

[2]STIKES Tujuh Belas, Karanganyar, Indonesia

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***Corresponding author:**
Annora Rizky Amalia

E-mail address:
annora@poltekindonusa.ac.id

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A B S T R A C T

Introduction: *Streptococcus mutans* is a bacteria that plays an important role in the occurrence of dental caries. Use of antibiotics to treat infections *Streptococcus mutans* often causes side effects. Cherry leaves (*Muntingia calabura* L.) contain active compounds that have the potential to have antibacterial effects. This research aims to determine the inhibitory power of cherry leaf ethanol extract against *Streptococcus mutans* in vitro. **Methods:** This research uses an experimental method with a post-test-only control group design. Cherry leaf ethanol extract was made by maceration method using 96% ethanol solvent for 5 days. The antibacterial test was carried out using the paper disc diffusion method with varying concentrations of cherry leaf extract, namely 20%, 40%, and 60% w/v dissolved in 10% DMSO. The positive control used was the amoxicillin antibiotic disc, while the negative control used 10% DMSO. **Results:** The results of the antibacterial test of the ethanol extract of cherry leaves in this study showed that at a concentration of 20% it had an average inhibitory power of 9.16 mm, the extract with a concentration of 40% had an inhibitory power of 11.33 mm, and at a concentration of 60% it had an inhibitory power of 12. 16mm. The positive control amoxicillin had an inhibitory power of 18.67 mm. **Conclusion:** Cherry leaf ethanol extract has potential as an alternative therapy to overcome *Streptococcus mutans* bacterial infections.

1. Introduction

Dental caries, a disease that attacks tooth structure and is characterized by damage to hard tooth tissue, is one of the most common dental and oral health problems throughout the world. It is estimated that 65% of children and 35% of adults worldwide experience dental caries. One of the main bacteria that plays a role in dental caries is *Streptococcus mutans.*

Streptococcus mutans is a facultative anaerobic gram-positive bacterium that can be found in dental plaque. These bacteria are able to produce lactic acid through carbohydrate fermentation, which then lowers plaque pH and causes tooth enamel demineralization. Demineralization of enamel is what ultimately triggers dental caries. The use of antibiotics to treat infections of *Streptococcus mutans* often causes side effects, such as antibiotic resistance, digestive disorders, and allergies. Therefore, safer and more effective alternative therapies are needed to treat infections of *Streptococcus mutans.*[1-3]

Cherry leaves (*Muntingia calabura* L.), a plant that is easily found in various regions in Indonesia, contains active compounds that have the potential to

have antibacterial effects. Several studies have shown that cherry leaf extract has antibacterial activity against various types of pathogenic bacteria, including *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*. Cherry leaves are rich in various active compounds, such as flavonoids, saponins and tannins. These compounds have various pharmacological effects, including antibacterial, anti-inflammatory, and antioxidant.[4-7] This research aims to determine the inhibitory power of cherry leaf ethanol extract against *Streptococcus mutans* in vitro.

2. Methods

The tools used in this study were test tubes, ose needle, bunsen, tweezers, sterile swabs, petri dishes, dropper pipettes, micro pipettes, tube racks, rulers, Erlemeyers, autoclaves, evaporator dishes, blenders, measuring cups, Becker glass, ovens, incubator, vacuum rotary evaporator, laminar air flow (LAF), analytical balance, mask, gloves, aluminum foil, filter paper, caliper, jar, and measuring flask. The materials used in this research were dried cherry leaves, *Streptococcus mutans*, ethanol 96%, Media MHA (Muller Hinton Agar), NA, NB, amoxicillin, aquadest, H_2SO_4, $FeCl_3$ 0,1%, magnesium, HCl 1%, NaCl 0,9%, Mc Farland solution, DMSO 10%.

The cherry leaves used are cherry plants obtained from Welar Pandeyan hamlet, Boyolali. The part used was the leaves, a sample of 900 grams of leaves that were still fresh and the leaves were perfectly green. Drying using an oven at 50°C for 5 days after drying resulted in 336 grams of dried simplicia. The dried leaves were then tested for drying shrinkage and water content on the simplicia using a moisture analyzer. Weigh a 300-gram sample of dry cherry leaves, grind them using a blender, then macerate them with 96% ethanol which can be dissolved in 3 liters for 5 days while stirring 1 x 24 hours, store the macerate in a cool place and not exposed to light. After the maceration results are separated using filter paper, it is then evaporated using a rotary evaporator at a temperature of 53°C and a water bath at a temperature of 50°C to produce a thick extract. Extracts from hard cherry leaves were then tested for water content using a tool moisture analyzer.

Muller Hinton Agar (MHA) media weighed 2.28 grams, added with 60 ml of distilled water, and heated until dissolved. Use an autoclave to sterilize the MHA media for 15 minutes at 121°C. Then, the media was placed in three petri dishes and left to solidify. Nutrient agar media weighs 0.56 grams, dissolves in 20 ml of distilled water, and heat until dissolved, then autoclave at 121°C for 15 minutes, pour into a 20 ml petri dish, and leave at room temperature until it solidifies. NA media is used for bacterial inoculation. The inoculation technique, also known as microbial cultivation, involves the transfer of sterile microorganisms from an old medium to a new medium with high precision. One cycle of *Streptococcus mutans* was taken, then streaked in a zig-zag manner on NA media, and then inoculated for one day at 37°C. Take 3-5 inoculated *Streptococcus mutans* colonies then the bacterial suspension is put into a test tube containing 3 mL of NB and incubated for 24 hours at 37°C to equalize the turbidity of the MC Farland solution.

MHA media that has been densely planted *Streptococcus mutans* bacteria using the streak plate method by means of a sterile swab dipped in a bacterial suspension whose turbidity level is adjusted to McFarland standards, the swab is lifted by pressing cotton on the tube wall, scratching the MHA media zigzag tightly in a circular manner on a petri dish, let stand for 5 minutes so that the bacterial suspension absorbs into the media. Then prepare a paper disc that has been soaked in several variations of extract concentration, namely 20%, 40%, and 60% which has been dissolved using 10% DMSO and let it sit for 20 minutes, then place the disc on the petri dish that has been marked. In this study, amoxicillin as a positive control and DMSO 10% as a negative control were used. The treated media was then incubated for 24 hours in an incubator at 37°C. Inhibition zone diameter data was measured and analyzed using descriptive statistics. Data were compared with positive controls and negative controls to determine the effectiveness of cherry leaf ethanol extract against *Streptococcus mutans*.

3. Results and Discussion

Table 1 shows that simplicia and cherry leaf extract have low water content, which reflects good product quality. The water content of cherry leaf simplicia in this study was 6.39%. This value is relatively low and indicates that the simplicia has been dried well. Simplicia with low water content is more durable and is not easily damaged by microorganisms. This is important to ensure the quality of simplicia during storage and distribution. The drying loss of cherry leaf simplicia in this study was 7.88%. This value indicates that the simplicia drying process has taken place effectively. High drying shrinkage indicates that a lot of water has been removed from the simplicia during the drying process. This is important to improve the quality of the simplicia and simplify the extraction process. The water content of cherry leaf extract in this study was 6.57%. This value is relatively low and indicates that the extraction process has gone well. Cherry leaf extract with low water content is more stable and not easily damaged. It is important to ensure the quality of cherry leaf extract during storage and use.

Table 1. Simplicia and extract parameter test results.

Parameter	Value (%)
Simplicia water content	6.39
Simplicia drying loss	7.88
The water content of cherry leaf extract	6.57

Table 2 shows that cherry leaves are rich in flavonoids, saponins, and tannins. Flavonoids are a group of polyphenolic compounds that play an important role in protecting the body from damage caused by free radicals. This compound has strong antioxidant properties, so it can help prevent various chronic diseases such as cancer, heart disease, and Alzheimer's. In the phytochemical test, cherry leaf extract showed a color change to orange when HCl, NaOH, and H_2SO_4 reagents were added. This shows that cherry leaves contain abundant flavonoids. Saponin is a steroid glycoside that has detergent properties. This compound can damage bacterial cell membranes, so it has a strong antibacterial effect. In phytochemical tests, cherry leaf extract showed stable foam formation when shaken with water. This shows that cherry leaves contain saponin which can help fight bacterial infections. Tannins are polyphenolic compounds that can bind proteins and form insoluble complexes. This compound has anti-inflammatory and antioxidant properties, so it can help reduce inflammation and protect the body from damage caused by free radicals. In phytochemical tests, cherry leaf extract showed a color change to blackish blue when reacted with $FeCl_3$ reagent. This shows that cherry leaves contain tannins which can provide various health benefits.

Table 2. Phytochemical test results of cherry leaves.

No	Compound	Cherry leaf extract	Results
1	Flavonoid	+	Orange
2	Saponin	+	Formed foam
3	Tannin	+	Blackish blue

Table 3 show that cherry leaf ethanol extract shows antibacterial effects against *Streptococcus mutans*. The inhibition zone, the area around the holes in the agar medium where bacteria do not grow, shows the effectiveness of the extract. The size of the inhibition zone increased with increasing extract concentration, indicating that its antibacterial effect was concentration-dependent. A concentration of 60% extract produced the largest inhibition zone, namely 12.16 ± 0.97 mm, indicating its potential as a

promising antibacterial agent. Amoxicillin, an antibiotic used as a positive control, produced a larger zone of inhibition compared to all concentrations of cherry leaf ethanol extract. This shows that amoxicillin has higher effectiveness in inhibiting the growth of *Streptococcus mutans*. Even though it has antibacterial effects, its potency still needs to be increased to match the effectiveness of conventional antibiotics. 10% DMSO, used as a negative control, did not produce an inhibition zone. It is important to point out that DMSO has no antibacterial effect and does not affect bacterial activity. This helps ensure that the antibacterial test results for cherry leaf ethanol extract are accurate and reliable. The ethanol extract of cherry leaves shows an antibacterial effect against *Streptococcus mutans*. The antibacterial effectiveness increased with increasing extract concentration. However, its potential still needs to be increased to match the effectiveness of conventional antibiotics.

Table 3. Comparison of inhibition zones between concentrations.

Cherry leaf ethanol extract concentration (% w/v)	**Inhibition zone (mm)**
20%	9,16 ± 0,56
40%	11,33 ± 0,87
60%	12,16 ± 0,97
Amoxicillin (positive control)	18,67 ± 1,32
DMSO 10% (negative control)	0

Flavonoids are a group of polyphenolic compounds that are widespread in nature and are found in various types of plants, such as fruits, vegetables, nuts, and seeds. This compound has a diverse chemical structure, with the characteristic feature of a flavone ring consisting of two aromatic rings connected by an oxygen bridge. Flavonoids have long been known for their various health benefits, including antioxidant, anti-inflammatory, and anticancer activity. In recent years, scientific research has increasingly shown that flavonoids also have promising potential as antibacterial agents. Flavonoids can interact with bacterial cell membrane phospholipids, causing membrane structure damage and intracellular leakage. This can disrupt the vital functions of bacteria and inhibit their growth. Flavonoids can inhibit the activity of enzymes involved in bacterial protein synthesis, such as DNA gyrase and RNA polymerase. Protein synthesis is essential for bacterial growth and reproduction, so its inhibition can significantly inhibit bacterial growth. Flavonoids can induce apoptosis, or programmed cell death, in bacteria. Apoptosis is the process of controlled self-destruction of cells and can be an important mechanism in fighting bacterial infections. Another study found that flavonoid extract from green tea leaves had strong antibacterial effectiveness against *Staphylococcus aureus* and *Escherichia coli*. Other research shows that the flavonoid apigenin can inhibit the growth of *Staphylococcus aureus* bacteria by interfering with protein synthesis. Another study also found that the flavonoid quercetin can induce apoptosis in *Escherichia coli* bacteria.[8-12]

Saponin is a steroid glycoside compound found in various plants, including cherry leaves (*Muntingia calabura* L.). This compound has a unique structure, with a sugar portion (glycoside) attached to the steroid framework. This structure gives saponins a variety of bioactive properties, including detergent effects and the ability to damage bacterial cell membranes. Saponins have detergent properties due to their molecular structure which consists of hydrophilic (glucose) and hydrophobic (steroid) parts. The hydrophobic part can interact with lipids in the bacterial cell membrane, causing disruption of membrane structure and permeability. This disturbance can cause leakage of important substances from within the bacterial cells, thereby inhibiting the growth and survival of the bacteria. Saponins not only disrupt the structure of bacterial

cell membranes, but can also directly damage them. This compound can form a complex with membrane lipids, thereby causing mechanical damage to the membrane. This damage can cause leakage of important proteins and enzymes from within the bacterial cells, thereby inhibiting the bacteria's vital functions and causing death. Apoptosis is a process of "programmed cell death" that occurs in eukaryotic cells, including bacteria. Saponin can induce bacterial apoptosis by activating the internal bacterial apoptosis signal pathway. This signaling pathway involves the activation of enzyme cascades and proteas that lead to DNA fragmentation and degradation of cellular proteins. Induction of apoptosis is another way saponins kill bacteria. Saponins can also inhibit the activity of important enzymes in bacterial metabolism. This compound can bind to enzymes and change their structure, thereby inhibiting the enzyme's function. Inhibition of this enzyme can disrupt various bacterial metabolic processes, such as respiration, protein synthesis, and DNA replication. A study shows that the ethanol extract of cherry leaves which is rich in saponins has a strong antibacterial effect against various pathogenic bacteria, including *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*. Other research identified the antibacterial mechanism of saponins, namely through disruption of bacterial cell membrane structure, induction of bacterial apoptosis, and inhibition of bacterial enzyme activity. Saponin shows potential as a natural antibacterial agent that is safe and effective for treating bacterial infections. Further research is needed to develop saponin formulations that are effective and safe for human use.[13-16]

Tannins, natural polyphenolic compounds found in various plants, have tremendous potential as antibacterial agents. Its ability to bind bacterial proteins and form insoluble complexes makes it a powerful weapon in fighting bacterial infections. Tannins have a high affinity for bacterial proteins, especially structural proteins and enzymes. When tannin binds to these proteins, it forms insoluble complexes, disrupting protein function and inhibiting bacterial metabolism. Tannins can also bind to bacterial membrane proteins, disrupting the transport of vital nutrients into cells. This causes nutritional deficiencies and starvation in the bacteria, thereby inhibiting their growth. Tannins can form a protective layer around bacterial cells, preventing the penetration of other antibacterials and strengthening bacterial resistance to drugs. A number of studies have proven the antibacterial effectiveness of tannin against various pathogenic bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*. Studies show that tannins from different plant sources, such as green tea, red grapes, and apple peels, have varying antibacterial effects. Green tea extract is rich in the tannin epigallocatechin gallate (EGCG), which has demonstrated strong antibacterial effectiveness against *Staphylococcus aureus* and *Escherichia coli*. EGCG can interfere with bacterial biofilm formation and inhibit bacterial growth. The tannins in red wine, especially resveratrol, have been shown to be effective in inhibiting the growth of the bacteria *Streptococcus pneumoniae*, which causes pneumonia. Resveratrol may also increase the effectiveness of conventional antibiotics. Apple peel extract is rich in tannin proanthocyanidin, which has demonstrated antibacterial effectiveness against *Salmonella typhi*, the cause of typhoid fever. Proanthocyanidin can interfere with bacterial motility and inhibit their invasion into host cells. Tannins have great potential to be developed as natural antibacterial agents in the pharmaceutical industry. Its ability to inhibit bacterial growth without significant side effects makes it a promising alternative to increasingly resistant conventional antibiotics. Although tannins show potential as effective antibacterial agents, several challenges still need to be overcome. One of the main challenges is ensuring the bioavailability of tannins, that is, their ability to be absorbed and transported to the site of infection. In addition, further research is needed to determine the optimal dosage and detailed mechanism of action of tannins. Tannin is a promising natural antibacterial compound with various advantages over conventional antibiotics. Further research is needed to optimize its potential and pave the way for the development of safe and effective natural antibacterial agents.[17-20]

4. Conclusion

The ethanol extract of cherry leaves shows an antibacterial effect against *Streptococcus mutans*. The antibacterial effectiveness increased with increasing extract concentration. However, its potential still needs to be increased to match the effectiveness of conventional antibiotics.

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