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Inhibitory test of turmeric leaves extract (*Curcuma Longa. L*) against the growth of *Streptococcus mutans* bacterial growth *in vitro*

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ABSTRACT. *Streptococcus mutans* is a bacterium that plays an essential role in forming dental caries. Odontogenic infection is an oral cavity infection that can develop from dental caries if not appropriately treated. Caries treatment can be done with the use of antimicrobial agents. The increasing resistance to antimicrobial drugs has made herbal medicines the choice because they are more effective and less harmful. The community uses turmeric leaves as a cooking spice and treatment for stomach aches in children. Turmeric leaf extract contains flavonoids, tannin, and phenolic antimicrobial active compounds. This research aims to find out if turmeric leaf extract inhibits the growth of the *Streptococcus mutans*. This study uses a post-test-only control condition approach in a laboratory setting. The concentration of turmeric leaf extract tested was 10%, 15%, and 20% with the positive control (Chlorhexidine 0,2% mouthwash) and negative control (DMSO) with five repetitions. Antibacterial effectiveness test using Kirby-Bauer disc diffusion method. The data analysis using ANOVA showed a p-value of 0.00 on inhibition, which indicates that turmeric leaf extract can suppress the development of *Streptococcus mutans*. The average inhibition zone obtained was 8.7 mm at a concentration of 10%, at a concentration of 15% at 9.5 mm, and at a concentration of 20% at 8.54 mm. This research concludes that turmeric leaf extract can inhibit the proliferation of *Streptococcus mutans* bacteria

KEYWORDS: Turmeric Leaf, *Streptococcus mutans*, Antibacterial

INTRODUCTION

Dental caries is a multifactorial disease that occurs due to the production of acid by bacterial fermentation of food debris on the tooth surface, causing damage to the hard tissues and demineralizing the teeth. The factors that cause caries are bacteria, time, host, and substrate.[1] *Streptococcus mutans* is the bacteria that contributes **the** most to tooth decay. *Streptococcus mutans* bacteria are Gram-positive bacteria with a cocci shape and are found in the flora normal of the oral cavity.[2]

Odontogenic infections can also occur due to the bacterium *Streptococcus mutans*. Odontogenic infection may be the initiation or continuation of pulp, periodontal disease, pericoronal disease, trauma, or postoperative infection. One of the leading causes of odontogenic infections is dental caries. However, odontogenic infections can be caused by exodontia, periodontal pockets, or pericoronitis. Odontogenic infections can usually start from dental or periodontal tissues and expand to complex anatomical structures in the oral cavity. When bacteria reach the tooth's pulp, necrosis will occur, which will induce abscess formation. The infection begins in the periapical tissue and continues across the periosteum of the cortical bone and over the pathway of least resistance.[3]

Streptococcus mutans bacteria can be found in the flora normal of the oral cavity, odontogenic infections, and dental caries. Bacteria found in the flora normal of the oral cavity can turn into pathogenic bacteria, causing odontogenic infections. *Streptococcus mutans* have a significant function in the formation of caries due to the carbohydrate fermentation process resulting in acid production and lowering the pH of the oral cavity, which causes tooth demineralization and infection. The infection can occur through several routes in the oral cavity, such as the pulpo periapical pathway. Bacteria can enter this pathway through the enamel, dentin, and pulp chambers to the apical area of the tooth. Infection in the pulpo periapical tissue begins with

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dental caries resulting from the invasion of *Streptococcus mutans* bacteria. Bacterial involvement in the periapical tissue can initiate an inflammatory response to the infected tissue. An abscess can form if the host conditions are not satisfactory and the virulence of bacteria is high enough in the infected areas.[4]

Various methods are used to prevent caries, odontogenic infection, and abscess formation by inhibiting the growth of caries-causing bacteria using herbal medicines. Herbal medicines are the choice because they increase efficiency and suppress dangerous traits, show low toxic effects on mammals, and are generally considered safe. Medicinal herbs such as turmeric leaf (*curcuma longa. l*) comes from the Zingiberaceae family and originates from South Asia with well-known records of use as medicine commodities. In addition, turmeric leaves are also known to be utilized in conventional cooking methods to add flavor and function as a food preservative.[5]

Secondary metabolites that act as antibacterial ingredients in turmeric leaves are flavonoids, tannins, and phenolic compounds. Flavonoid compounds and tannins can prevent spoilage, also **known as** antibacterial.[6] The flavonoid bioactive compounds found in turmeric leaves have antibacterial properties through complex compounds that form to attack extracellular proteins and degrade the membrane cells' integrity by dissolving the membrane, thus damaging the cell membrane and releasing its intracellular components—followed by cell death. The active compounds of tannin also show antibacterial properties utilizing protein deposition because it is suspected that tannins have the same effect as phenolic compounds. Tannins can cause bacterial lysis by forming complexes with polypeptide proteins in the bacterial cell wall, causing disruption of the cell wall. In addition to harming cell membranes, phenolic substances can modify the permeation walls, preventing or speeding up cellular proliferation.[7]

MATERIALS AND METHODS

This study was carried out in vitro in an experimental laboratory setting at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, University of North Sumatra, and at the Research and Development Laboratory of Medicinal Plants ASPETRI (Association of Indonesian Traditional Herbs Medicine). The research period was approximately two months, from February to April 2022.

The samples used in this research were turmeric leaves grown at the Tasbi Complex 2 block VI no. 57, which were studied as fresh leaves. Extraction of manufacture began with the manufacturing of Simplicia. Turmeric leaves were cut and dried in the drying cabinet, then mashed to obtain Simplicia powder.

Extracts were made by the maceration method. Simplicia was then immersed in 96% ethanol solvent for 24 hours, then filtered to obtain the first macerate. Then re-immersed with 96% ethanol and filtered to obtain a second macerate. The two macerates were combined and put into a rotary evaporator to obtain a thick extract of turmeric leaves, followed by the manufacture of turmeric leaf extract with various concentrations of 10%, 15%, and 20%.

Streptococcus mutans bacteria were planted in nutrient agar media and incubated at 37°C for 24 hours. Then, the bacterial suspension was taken in streaks on MHA media using a sterile cotton swab. An inhibition test was performed using the Kirby-Bauer method using disc paper with 10%, 15%, and 20% turmeric leaf extract, positive control (chlorhexidine 0.2%), and negative control (DMSO) on the media. Each concentration was repeated five times. Incubation was carried out at 37°C for 24 hours. The inhibition zone observed was measured with a caliper.

RESULTS

The study findings showed that turmeric leaf extract concentrations of 10%, 15%, and 20% and positive control (0.2% chlorhexidine) indicated the formation of an inhibitory zone. In contrast, the negative control (DMSO) did not show an inhibition zone. The inhibition zones formed at concentrations of turmeric leaf extract 10%, 15%, 20%, and 0.2% chlorhexidine against *Streptococcus mutans* were 8.7±0.45 mm; 9.5±0.58mm; 8.54±0.42mm; 14.42±1.40mm. In contrast, the negative control (DMSO) did not form a diameter of the inhibition zone.

The observation of the diameter of the inhibition zone showed the strength of the inhibition against *Streptococcus mutans* bacteria categorized according to Davis & Stout (1976), shown in Table 1. The power of the diameter of the inhibition zone at concentrations of 10%, 15%, and 20% was in the moderate category. In comparison, the strength of the diameter of the 0.2% chlorhexidine inhibition

zone as a positive control was classified as strong. Table 2. Shows the inhibition of *Streptococcus mutans*

Table 1. Inhibitory Zone Diameter Category[8]

Antibacterial Activity	Inhibition zone diameter (mm)
Weak	<5
Average	5-10
Strong	10-20
Very Strong	>20

Table.2 Diameter of inhibition zone of turmeric leaf extract (*Curcuma Longa.l*) against Streptococcus mutans

Groups	Inhibition zone diameter (mm)	Antibacterial strength
10% Turmeric leaf Extract (*Curcuma Longa. L*)	8.7	Average
15% Turmeric leaf Extract (*Curcuma Longa. L*)	9.5	Average
20% Turmeric leaf Extract (*Curcuma Longa. L*)	8.54	Average
Control positive (chlorhexidine 0,2%)	14.42	Strong
Control negative (DMSO)	0	Weak

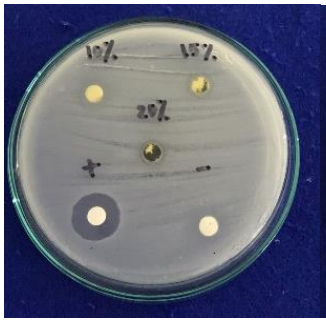

Figure 1. Repetition 1 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

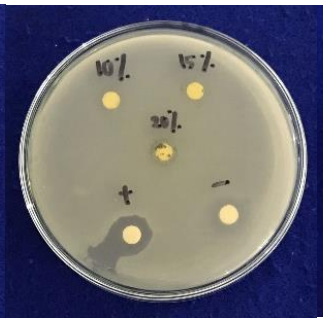

Figure 2. Repetition 2 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

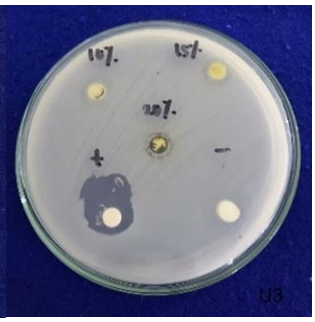

Figure 3. Repetition 3 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

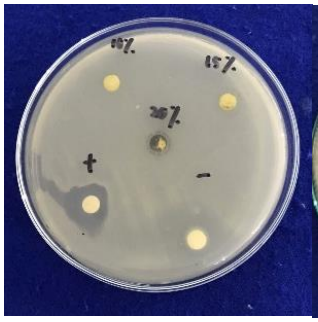

Figure 4. Repetition 4 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

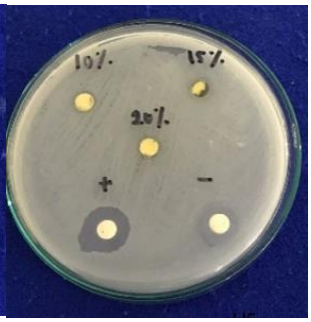

Figure 5. Repetition 5 of turmeric leave extract 10%, 15% and 20%, control positive and control negative

DISCUSSION

The best treatment results in the inhibition test of turmeric leaf extract against *Streptococcus mutans* bacteria was at a concentration of 15% with an average inhibitory diameter of 9.5 mm. The 0.2% chlorhexidine mouthwash formula was used as a positive control with an average diameter of inhibition of 14.42 mm. When compared with the positive control treatment, the results showed the best treatment was turmeric leaf extract with a concentration of 15%. Still, it could not exceed the antibacterial activity of the 0.2% chlorhexidine mouthwash formula.

The existence of the inhibitory ability of turmeric leaf extract on the growth of *Streptococcus mutans* bacteria in vitro proves that there are compounds that function as bacteriostatic agents in the extracts that play a role in inhibiting the growth of the bacteria tested. The active substance of phenol in turmeric leaf extract has bacteriostatic properties. Phenol is an antimicrobial agent that denatures proteins and damages cell membranes. Antibacterial compounds can interact with the cell walls of bacteria, causing a decrease in the permeability of bacterial cells, which diffuses into the cells causing inhibition of bacterial growth. It shows the bacteriostatic properties of turmeric leaf extract.[9]

Streptococcus mutans is a gram-positive bacterium. There are differences between the structure of the cell walls of gram-positive and gram-negative bacteria that can affect the sensitivity towards antibacterial agents, thus showing different results. In gram-positive bacteria, a thicker cell wall is constructed of approximately 40 layers of peptidoglycan. The peptidoglycan reaches 70% of the cell wall's dry mass, causing the cell wall to become thick and stiff. Meanwhile, in gram-negative bacteria, the wall thickness is thinner, about 10% of the dry mass of the cell wall. The lipid content in gram-negative bacteria is high. Thus, it has a protein porin that acts as a channel for the entry of active substances into bacterial cells. The permeability of the active substance increases with high levels of lipids in the cells. It allows the active substance to enter the cell, causing cell damage which impairs enzymes' activity in the cell.[10]

The temperature is a factor that can affect the quality of the extract. Extract storage temperature can affect the content of active compounds in the extract. The extraction storage at higher temperatures decreases the range of active compounds in the extract. Meanwhile, storage of the extract at a low temperature, between 2-5^{o}C, can maintain the content of chemical compounds in the extract from decreasing.[11] In conclusion, the long storage time of the extract can affect its strength. Storage of sections for more than seven days can cause an increase in the activity of microorganisms that will lead to spoilage of the extract..

The purpose of this study is to continue research by making turmeric leaf extract as a mouthwash, asepsis, or antibiotics. It is because turmeric leaf extract has compounds that have the potential as antibacterial ingredients. Secondary metabolites such as saponins, tannins, terpenoids, triterpenoids, and flavonoids are polar so that they can inhibit bacterial growth by denaturing proteins in bacterial cells. Turmeric leaf extract is an antibacterial material suitable for aseptic ingredients such as mouthwash, asepsis ingredients, and antibiotics. It is proven according to previous research, which showed the antibacterial properties of turmeric leaves after being used as a hand sanitizer and food preservative.

This study proves that turmeric leaf extract has an antibacterial effect on the growth of *Streptococcus mutans* bacteria in the oral cavity in vitro. It is proven by the presence of an inhibition zone (clear zone) around the paper disc. This result is the first step in the possibility of using turmeric leaf as an alternative antibacterial ingredient in dentistry. Turmeric leaves are good antibacterial ingredients because turmeric leaves can help accelerate wound healing. In addition, turmeric leaf can also be used as a food preservative due to its strong antibacterial properties. In daily life, turmeric leaves are usually used as a cooking spice and medicine for stomach aches in children. The results of this study indicate that turmeric leaves have moderate antibacterial power. There is antibacterial potential against *Streptococcus mutans* because turmeric leaves have flavonoid, tannin, and active phenolic compounds that have antibacterial activity so they can be used as a basis for research. It shows turmeric leaves' antibacterial properties, which is suitable for further study as an antiseptic ingredient such as mouthwash.

CONCLUSION

Turmeric leaf extract (*Curcuma Longa. L*) can restrict the development of *Streptococcus mutans* bacteria in vitro. At a concentration of 15%, turmeric leaf extract (*Curcuma Longa. L*) had the largest inhibition zone in restricting the development of *Streptococcus mutans* bacteria in vitro, with an average inhibition diameter of 9.5 mm.

Recommendation

1. Further research is needed on the antibacterial effect of turmeric leaf extract (*Curcuma Longa. L*) in dosage form so that it can be used in treating dental and oral diseases.
2. Further research is needed on the antibacterial effect of turmeric leaf extract (*Curcuma Longa. L*) in dosage form so that it can be used in treating dental and oral diseases.
3. Further research is needed for antibacterial tests with other methods so that the values of the Minimum Inhibitory Concentration (MIC) and the minimum bactericidal concentration (MBC) of turmeric leaf extract (*Curcuma Longa. L*) can be calculated on the growth of *Streptococcus mutans* bacteria.
4. It is necessary to carry out an antibacterial test with other extraction methods against other gram-positive and gram-negative bacteria.
5. It is necessary to do an antibacterial test of turmeric leaves using another formula for determining sample size to prevent bias.
6. Further research is needed on turmeric (*Curcuma Longa. L*) leaves as asepsis, mouthwash, and antibiotics.

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