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Antimicrobial Assessment of *Curcuma longa* L Against *Streptococcus mutans* Growth

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ABSTRACT

Background. *Streptococcus mutans* is the most common oral endogenous microorganism found at the beginning of plaque formation until the formation of dental caries. If not handled properly, caries can lead to odontogenic infections. Increased resistance to antimicrobial drugs has made herbal ingredients an alternative treatment because they are more effective and do not cause side effects. This turmeric leaf contains active antimicrobial compounds of flavonoids, tannins, and phenolics. **Objective**. This study aimed to determine the effectiveness of turmeric leaf extract (*Curcuma longa*. L) as an antimicrobial on the growth of *Streptococcus mutans* bacteria. **Materials and Methods**. 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 serial dilution method. **Results**. The data analysis using the Oneway ANOVA test showed a p-value <0.05, meaning that the number of *Streptococcus mutans* bacterial colonies in both the treatment and control groups had a significant difference in number. The antibacterial effect at a 10% concentration of turmeric leaf extract (*Curcuma longa*. L) with an average and standard deviation of 45.20 ± 3.83, at a 15% concentration of 43.80 ± 3.11, at a 20% concentration of 23.60 ± 12.30. **Conclusion**. This study concludes that turmeric leaf extract (*Curcuma longa*. L) has antibacterial effectiveness against *Streptococcus mutans* bacteria.

Keywords: *Curcuma longa* L**,** Turmeric Leaf, *Streptococcus mutans*, Antibacterial

ABSTRAK

Latar belakang: *Streptococcus mutans* merupakan mikroorganisme endogen rongga mulut yang paling banyak ditemukan pada awal pembentukan plak hingga terbentuknya karies gigi. Jika tidak ditangani dengan baik, karies dapat menyebabkan infeksi odontogenik. Peningkatan resistensi terhadap obat antimikroba menjadikan bahan herbal sebagai pengobatan alternatif karena lebih efektif dan tidak menimbulkan efek samping. Daun kunyit ini mengandung senyawa antimikroba aktif flavonoid, tanin dan fenolat. **Tujuan**. Tujuan penelitian ini adalah untuk mengetahui efektivitas ekstrak daun kunyit (*Curcuma longa*. L) sebagai antimikroba terhadap pertumbuhan bakteri *Streptococcus mutans*. **Bahan dan Metode**: Penelitian ini menggunakan pendekatan *post-test-only control condition* di laboratorium. Konsentrasi ekstrak daun kunyit yang diuji adalah 10%, 15%, dan 20% dengan kontrol positif (obat kumur Chlorhexidine 0,2%) dan kontrol negatif (DMSO) dengan lima kali pengulangan. Uji efektivitas antibakteri menggunakan metode pengenceran serial. **Hasil**: Hasil analisis data dengan menggunakan uji Oneway ANOVA menunjukkan p-value < 0,05 artinya jumlah koloni bakteri *Streptococcus mutans* baik pada kelompok perlakuan maupun kontrol memiliki perbedaan jumlah yang bermakna. Efek antibakteri ekstrak daun kunyit (*Curcuma longa*. L) konsentrasi 10% dengan rata-rata dan standar deviasi 45,20 ± 3,83, pada konsentrasi 15% sebesar 43,80 ± 3,11, pada konsentrasi 20% sebesar 23,60 ± 12,30. **Kesimpulan**: Kesimpulan dari penelitian ini adalah ekstrak daun kunyit (*Curcuma longa*. L) memiliki efektivitas antibakteri terhadap bakteri *Streptococcus mutans*.

Kata Kunci: Daun Kunyit, *Streptococcus mutans*, Antibakteri

1. Introduction

Dental caries is one of the most common dental and oral diseases in society, which is a disease of the hard dental tissues caused by microorganisms, one of which is *Streptococcus mutans* in fermented carbohydrates.[1] Apart from being the primary agent causing caries, *Streptococcus*

mutans can also develop into the periapical tissue to cause tooth pain, which then continues to cause periapical abscesses, periapical granulomas, and odontogenic infections.[2]

Odontogenic infection is the most common oral infection influenced by a mixture of aerobic and anaerobic bacteria. Odontogenic infection may be the initiation or continuation of pulpal, periodontal, pericoronal disease, trauma, or postoperative infection. Odontogenic infection can start from the tooth or periodontal tissue, spreading to deep anatomical structures. If bacteria reach the tooth's pulp, they will enact necrosis and induce abscess formation.[3]

Streptococcus mutans is a gram-positive cocci-shaped, non-motile bacteria; some are members of the normal flora in the oral cavity. *Streptococcus mutans*, which is the dominant bacterium in caries formation, is also a bacterium that resides in the normal flora of the oral cavity, which can play a role in the process of fermenting carbohydrates to form acids which can cause tooth demineralization and form an infection in the oral cavity.[4] The disease can occur through several routes, including the pulp-periapical path. This pathway is the entry route for *Streptococcus mutans* bacteria through the enamel, dentine, and pulp chamber to the apical of the tooth. Infection of the pulp-periapical tract is common and usually begins with the formation of dental caries caused by the invasion of *Streptococcus mutans* bacteria.[5]

Various preventive treatments are carried out to reduce oral bacteria, namely by minimizing the growth of pathogenic bacteria themselves. Natural products such as herbal ingredients from plant extracts can mitigate the development of oral bacteria. The use of herbal products as medicinal ingredients is currently increasingly being chosen by the wider community.[6] Generally, diseases caused by bacterial infections can be treated by using antibiotics. Still, using antibiotics for a long time will cause resistance to bacteria and can cause side effects for users, for example, allergic, toxic reactions and biological and metabolic changes. Therefore, many herbal medicines have recently been developed as an alternative to antibiotics to treat diseases, especially those caused by bacterial infections.[7]

Turmeric leaves (*Curcuma longa.* L) are a spice for several dishes. Hence, using turmeric leaves is still limited and even becomes a waste. Turmeric leaves contain chemical compounds in the form of bioactive compounds such as phenols, flavonoids, and tannins.[8] The content of these secondary metabolites has polar and nonpolar groups that are active in inhibiting bacterial growth by denaturing proteins, changing membrane permeability, inhibiting the action of enzymes, and damaging the cell walls of bacteria such as *Streptococcus mutans, Staphylococcus aureus,* and *Escherichia colli*.[9] Besides that, turmeric leaves contain essential oils of *monoterpene, sesquiterpene, diterpene, polyterpene, aldehyde, alcohol, ester, ether,* and ketone groups. In addition, the essential oil components that have been identified include *δ-limonene, a-pinene,* and *myrcene*.[10] The *δ-limonene* compound is anti-carcinogenic by preventing the formation of carcinogenic compounds and suppressing tumor growth. The presence of *β-pinene, 1,8-cineol,* and *terpinen* compounds have bacteriostatic properties.[11]

2. Material and Methods

This study was conducted 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 October 2022 to January 2023.

2.1. Extract Preparation

The samples used in this research were turmeric leaves grown at Sei Tembo Kuala Subdistrict – Langkat Regency, 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%.[12]

2.2. *Antibacterial Assay*

Streptococcus mutans bacteria were planted in blood agar media and incubated at 37°C for 24 hours. Then, the bacterial suspension was taken, and the dilution process was carried out using the serial dilution method in five tubes containing 0.9% NaCl, planted in MHA media, and incubated at 37°C for 24 hours. The bacterial suspension was taken in streaks on MHA media using an inoculating loop. An inhibition test was performed using the serial dilution method with 10%, 15%, and 20% turmeric leaf extract, positive control (chlorhexidine 0.2%), and negative control (DMSO) on the media.[13]

After that, prepare five tubes and label them for each extract concentration, namely 10%, 15%, and 20%, positive and negative control. Take as much as 3.5 ml and put it in each tube that has been labeled mark and mixed with 0.5 ml of bacterial suspension, which has passed the serial dilution method and vortexed until homogeneous. Then 0.1 ml of the rest was taken using an Eppendorf pipette and dripped onto MHA media, leveled using the scatter method, incubated for 24 hours, then observed and counted the number of bacterial colonies growing on the media using a colony counter.

2.3. *Data Analysis*

Colony inhibition of S. mutans was analyzed using the Oneway ANOVA test showed a p-value <0.05.

3. Result and Discussion

The study findings have been done, and there is a significant difference in turmeric leaf extract concentrating 10%, 15%, and 20%, 0.2% chlorhexidine (positive control) and DMSO (negative control). This indicates that turmeric leaf extract (*Curcuma longa*. L) has antibacterial effectiveness against *Streptococcus mutans* bacteria. The results of calculating the number of bacteria with a strong antibacterial ability to inhibit can be seen in 20% turmeric leaf extract and 0,2% chlorhexidine, shown in Table 1.

Based on the results of the Oneway ANOVA test of turmeric leaf extract (*Curcuma longa*. L) with a concentration of 10%, 15%, and 20%, 0.2% chlorhexidine (positive control) and DMSO (negative control) showed a significant value where p = 0.000 Based on the significance value, it can be stated that there is a significant difference in the number of bacterial colonies between turmeric leaf extract concentrating 10%, 15% and 20%, 0.2% chlorhexidine (positive control) and DMSO (negative control), this indicates that the leaf extract turmeric (*Curcuma longa.* L) has antibacterial effectiveness against *Streptococcus mutans* bacteria, shown in Table 2.

Table 1. Calculate the bacterial colonies from 10%, 15%, 20% turmeric leaf extract, 0.2% chlorhexidine (positive control), and DMSO (negative control).

Repetition	Groups				
	10%	15%	20%	K+	K-
1	40	39	38	6	38
2	50	44	9	13	34
3	43	46	15	21	32
4	47	47	22	12	37
5	46	43	34	17	36

Table 2. One Way ANOVA analysis of 10%, 15%, and 20% turmeric leaf extract, 0.2% chlorhexidine (positive control), and DMSO (negative control)

Groups	N	Mean ± SD	P Value
10%	5	45,20 ± 3,83	
15%	5	43,80 ± 3,11	
20%	5	23,60 ± 12,30	0,000
Khlorheksidin 0,2% (Control Positive)	5	13,80 ± 5,63	
DMSO (Control Negative)	5	35,40 ± 2,40	

The best treatment results in the effectiveness test of turmeric leaf extract against *Streptococcus mutans* bacteria were at a concentration of 20% with an average value of 23.60. The 0.2% chlorhexidine mouthwash formula as a positive control had an average value of 13.80. When these

results were compared to the positive control treatment group, the best treatment groups with a concentration of 20%. Still, it could not exceed the antibacterial activity of the 0.2% chlorhexidine mouthwash formula. If you look at the overall data, it shows that the active ingredient in turmeric leaf extract can inhibit the growth of *Streptococcus mutans* bacteria. In 0.2% chlorhexidine, an ionic bond attracts cations from the chlorhexidine molecule and the cell wall anions of *Streptococcus mutans*.[14] This ionic bond will cause a selective increase in peptidoglycan permeability of *Streptococcus mutans,* so the cell membrane becomes damaged, and leaky cytoplasm eventually causes bacterial death.[15]

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.[16] Turmeric leaves (*Curcuma longa.* L) work as an antibacterial by forming complex compounds against extracellular proteins that prevent the integrity of the bacterial cell membrane and dissolve it will damage the cell membrane, followed by the release of intracellular compounds and cause cell death. This happens because of active flavonoid compounds in turmeric leaves (*Curcuma longa.* L).[16] Then, the presence of tannins in turmeric leaves (*Curcuma longa.* L) results in a protein deposition process that causes bacterial lysis by forming complexes with bacterial cell wall polypeptide proteins, disrupting the bacterial cell wall. The phenolic compounds can also damage cell membranes, resulting in changes in cell wall permeability, inhibiting cell growth until cell death.[16]

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. 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.[17]

Several factors can affect the results of calculating the number of bacterial colonies between the dilution series and the method or culture technique used. Using serial dilution can reduce the density of bacterial colony growth from the sample. The culture method used when inoculating the sample after serial dilution is the spread plate method with dry glassy or what is known as the triangle.[18] The bacterial colony method cultured using a spread plate with dry glassy had large colony sizes and tended to cluster or agglomerate, in contrast to the modified results of the spread cup method using round plates which had smaller and separate colony sizes.[19] This can affect the results of calculating the number of growing bacterial colonies because the colony count results in the spread plate method with Dryglasky are less than the scatter plate method using round loops.[20]

This study proves that turmeric leaf extract has an antibacterial effect on the growth of *Streptococcus mutans* in the oral cavity in vitro. This was proven by the difference in the number of bacterial colonies on the agar media filled with turmeric leaf extract and planted with *Streptococcus mutans* bacteria. This result is the first step towards achieving the ultimate goal of research utilizing turmeric leaves as an alternative antibacterial ingredient in dentistry.[21] Turmeric leaves are good antibacterial ingredients because they can help speed up wound healing. In addition, in previous studies, it was found that turmeric leaves were proven to be used as food preservatives and hand sanitizers because of their strong antibacterial properties, so this was a good discovery for further research to be carried out so that they could be developed into antiseptic ingredients such as mouthwashes.[16]

4. Conclusion

The in vitro study demonstrates the antimicrobial properties of turmeric leaf extract (Curcuma longa. L) against the growth of *S. mutans* in the oral cavity. In vitro, it has been observed that a concentration of 20% of turmeric leaf extract (*Curcuma longa*. L) exhibits the most potent antibacterial activity against *S. mutans*, in comparison to varying concentrations of turmeric leaf extract.

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Authors Contribution

Contribution	Dohude G A	Isnandar I	Siregar I B	Tanzia A
Concepts or ideas	V	V	V	V
Design	V	V		V
Definition of intellectual content	V		V	V
Literature search	V	V		V
Experimental studies	V		V	
Data acquisition		V		V
Data analysis	V		V	
Statistical analysis		V		V
Manuscript preparation	V	V	V	V
Manuscript editing	V	V		V
Manuscript review	V	V	V	V

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