

Effectiveness of *Curcuma longa* L on the growth Inhibition of *Streptococcus sanguinis*

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ABSTRACT

Background: A dry socket is a severe pain one to three days after tooth extraction. *Streptococcus sanguinis* (*S. sanguinis*) is reported to inhibit the healing process of dry sockets. *Curcuma longa* L contains flavonoids, tannins, saponins, and alkaloids which have the potential as antibacterials. **Objective.** This study evaluated the antibacterial properties of *Curcuma longa* L. on the *S. sanguinis*. **Materials and Methods:** This experimental study used a post-test-only control group design. Turmeric leaf extract is made by using the maceration extraction method. The method used in the inhibition test used Kirby-Bauer disc diffusion with five samples for each treatment. The sample consisted of five treatment groups: turmeric leaf extract with concentrations of 10%, 15%, and 20%, and positive control (clindamycin) and negative control (DMSO) groups. Data were analyzed using the One Way ANOVA and Post Hoc tests. **Results.** The results of data analysis using ANOVA showed a p-value of 0.00 on inhibition, which means that turmeric leaf extract can inhibit the growth of *S. sanguinis*. The average inhibition zone obtained was 9.52 mm at a concentration of 10%, at a concentration of 15% at 9.84 mm, and at a concentration of 20% at 10.18 mm. **Conclusion:** *Curcuma longa* L extract inhibited the growth of *S. sanguinis* on a moderate scale. Higher concentrations showed the best inhibition values.

Keywords: Turmeric Leaf, *S. sanguinis*, Antibacterial

ABSTRAK

Latar belakang: *Dry socket* adalah nyeri hebat yang terjadi satu sampai tiga hari setelah pencabutan gigi. *S. sanguinis* dilaporkan dapat menghambat proses penyembuhan *dry socket*. *Curcuma longa* L mengandung flavonoid, tanin, saponin dan alkaloid yang berpotensi sebagai antibakteri. **Tujuan:** Penelitian ini bertujuan untuk melihat bagaimana aktivitas antibakteri dari masing-masing konsentrasi ekstrak daun kunyit (*Curcuma longa* L.). **Bahan dan Metode:** Penelitian ini merupakan penelitian eksperimen dengan menggunakan *post test only control group design*. Ekstrak daun kunyit dibuat dengan menggunakan metode ekstraksi maserasi. Metode yang digunakan dalam uji daya hambat menggunakan difusi cakram Kirby-Bauer dengan 5 sampel untuk tiap-tiap perlakuan. Sampel terdiri dari lima kelompok perlakuan yaitu ekstrak daun kunyit dengan konsentrasi 10%, 15%, 20% dan kelompok kontrol positif (klindamisin) dan kontrol negatif (DMSO). Data dianalisis dengan uji One Way Anova dan uji Post Hoc. **Hasil:** Hasil analisis data menggunakan ANOVA menunjukkan nilai p-value 0,00 terhadap daya hambat yang menunjukkan bahwa ekstrak daun kunyit mampu menghambat pertumbuhan bakteri *S. sanguinis*. Rata-rata zona hambat yang diperoleh sebesar 9,52 mm pada konsentrasi 10%, pada konsentrasi 15% sebesar 9,84 mm, dan pada konsentrasi 20% sebesar 10,18 mm. **Kesimpulan:** Ekstrak *Curcuma longa* L mampu menghambat pertumbuhan *S. sanguinis* dengan skala sedang, konsentrasi yang lebih tinggi menunjukkan nilai hambat yang terbaik.

Kata Kunci: Daun Kunyit, *S. sanguinis*, Antibakteri

1. Introduction

Ideal tooth extraction is the painless removal of a tooth or tooth root with minimal trauma to the supporting tissues so that the socket after extraction can heal usually and does not cause postoperative prosthetic complications.¹ One of the post-extraction complications is a dry socket. A dry socket is severe pain one to three days after tooth extraction. This is caused due to the opening of the socket on the tooth that is released.² The pathogenesis of dry sockets can be explained by increased fibrinolytic activity in or around the tooth socket causing partial or complete lysis and

destruction of the blood clot. This activity can be caused by enzymes produced by bacteria that attack the wound retraction or tissue kinases released during inflammation.³ The most common bacteria associated with dry socket infections, one of the common complications of post-extraction cases is the *Streptococcus sp* group such as *Streptococcus viridans* (*S. viridans*).⁴

S. sanguinis is one of the bacteria from the *S. viridans* group, which is found in the oral cavity. This facultative anaerobic Gram-positive bacterium is an ordinary member of the microflora in the human oral cavity, where these bacteria can inhibit the healing process of dry sockets. For this reason, infection prevention can be done by giving antibiotics.⁵ Topical and systemic antibiotics can reduce the incidence of dry sockets by preventing the growth of these bacteria. Antibiotics such as clindamycin and amoxicillin effectively treat infections associated with dry sockets.⁶ However, despite the superiority of antibiotics, they have drawbacks, including causing resistance. This research was carried out to find drugs to replace antibiotics, one of which was switching to medicinal plants.⁷

Turmeric leaves are one of the medicinal plants that can be used as an antibacterial. People in the areas of the islands of Sumatra and Java commonly use turmeric leaves. Turmeric leaves are widely used for dishes such as curry, because they can eliminate the stinky smell and add to the dish's aroma. Thin slices of turmeric leaves are widely used as a mixed ingredient for cooking chips and peek to make the perfume fragrant.⁷

A previous study by Eris Septiana and Partomuan Simanjuntak in 2015 proved that turmeric leaves have good antibacterial activity.⁸ Research conducted by Ilham stated that turmeric leaves subjected to the ethyl acetate phytochemical test contained flavonoids, triterpenoids, saponins, glycosides, tannins, and alkaloids.⁹ In a study conducted by Usamah et al. in 2021, it was found that turmeric leaves can inhibit *Staphylococcus aureus* bacteria.¹⁰ Based on research conducted by Garcia-Gomes et al. in 2012, it was found that turmeric leaves could inhibit the growth of *Candida albicans*.¹¹

Based on the description above, this research will test the effectiveness of turmeric leaf extract (*Curcuma Longa* L.) against *S. sanguinis*. This study aims to see how the antibacterial activity of each concentration of turmeric leaf extract (*Curcuma longa* L.) so that it is hoped that in the future, the results of this study can be developed into natural antibiotics derived from the turmeric plant.

2. Material and Methods

This experimental laboratory research has a post-test-only control group research design. This research was conducted to test the inhibitory power of turmeric leaf extract (*Curcuma longa* L.) against *S. sanguinis* in vitro. The study was conducted at the ASPETRI Medicinal Plant Research and Development Laboratory (Indonesian Herb Traditional Medicine Association) and Pharmaceutical Microbiology Laboratory, University of North Sumatra, from October 2022 to January 2023.

This study used five treatment groups of turmeric leaf extract concentrations of 10%, 15%, 20%, clindamycin as the positive control group and DMSO as the negative control group. Each concentration of turmeric leaf, clindamycin, and DMSO used in this study was repeated five times. The materials used included: turmeric leaves (*Curcuma longa* L.) obtained from the garden at the ASPETRI Medicinal Plant Research and Development Laboratory (Association of Indonesian Traditional Medicine Medicines), the test bacteria (*S. sanguinis*) obtained from the Microbiology Laboratory of the University of North Sumatra Hospital, clindamycin, DMSO, Mueller Hinton Agar, Aquades and 96% ethanol.

2.1. Extract Preparation

The extraction method used in this research is cold extraction by maceration. Maceration is an immersion method, carried out by immersing the sample powder in a solvent with a ratio of 1:10. As much as 500 mg of turmeric leaf *Simplicia* was soaked in 1000 ml of 96% ethanol, soaked for 6 hours while stirring, then left for 18 hours. This maceration results in the first liquid extract being filtered using filter paper. The dregs or sediment resulting from the first liquid extract was macerated again by soaking it for 24 hours in 500 ml of 96% ethanol, after which it was filtered again. The result of this maceration is the second liquid extract. The first liquid extract is combined with the second and evaporated or concentrated to get a 100% viscous extract using a rotary

evaporator at 40°C. Then the thick extract obtained was diluted into each concentration of 10%, 15%, and 20%.¹²

2.2. Antibacterial assay

The antibacterial effect of turmeric leaf extract was carried out using the Disk Diffusion method (Kirby-Bauer test) with Mueller Hilton agar media. Antimicrobial substances are saturated into paper disks (paper discs). The paper discs are then placed onto solid agar media inoculated with the bacteria to be tested. Solid agar media was then incubated for 24 hours at 37°C. After 24 hours of incubation, observations were made of the inhibition zones formed and measured using calipers.¹³

2.3. Data analysis

The effect of antibacterial of *S. sanguis* was analyzed by One Way ANOVA and Post Hoc test. Also, the Significance is $p < 0.05$

3. Result and Discussion

From the observations that have been made, it was found that there was a clear zone around the disc paper, which had previously been soaked using turmeric leaf extract with a concentration of 10%, 15%, and 20% and clindamycin solution, which showed inhibition of the growth of the *S. sanguinis* bacteria with different diameters of the clear zone formed. Meanwhile, around the paper disk that was soaked using DMSO, no clear zone formed, indicating no inhibition of the growth of *S. sanguinis* bacteria because the DMSO solution did not have the antibacterial ability.

Table 1. Inhibitory effect of *Curcuma longa* L on the growth of *S. sanguinis*

Groups	Repetition (mm)					Average diameter (mm)	SD
	1	2	3	4	5		
C10%	9,8	9,6	9,2	9,8	9,2	9,52	0,3
C15%	10,1	9,9	9,7	10	9,5	9,84	0,24
C20%	10,4	10,2	10	10,3	10	10,18	0,18
Positive Control	38,7	38,7	38,5	39,2	39,2	38,86	0,32
Negative Control	0	0	0	0	0	0	0

Positive Control (Clindamycin); Negative Control (DMSO)

Data from the antibacterial effectiveness test results of turmeric leaf extract (*Curcuma longa* L.) were then tested for normality using the Shapiro-Wilk test. The Shapiro-Wilk test was chosen because the number of samples in this study was less than 50. The normality test results indicated that all treatment groups had standard data. The clindamycin-positive control group had $p = 0.147$, the 10% extract treatment group had $p = 0.086$, the 15% extract treatment group had $p = 0.787$, and the 20% extract treatment group had $p = 0.377$. All treatment groups are said to have standard data because the significance value is $p > 0.05$, so it can be concluded that the research data is usually distributed.

Based on the test results carried out using the one-way ANOVA test, it was obtained $p = 0.000$ ($p < 0.05$), which means that there is a significant difference in the diameter of the inhibition zone of turmeric leaf extract with concentrations of 10%, 15%, 20%, positive control, and negative control. Thus, H_0 is rejected, and the conclusion is H_a is accepted. These results show that turmeric leaf extract (*Curcuma longa* L.) has an antibacterial effect to inhibit the growth of *S. sanguinis*. The results of the LSD Post Hoc test analysis in this study showed an asterisk (*) in all groups which explained that each treatment group had a significant difference from the other groups. The three concentrations of the extracts used showed differences in each group, and it was proven that 20% turmeric leaf extract had the highest antibacterial activity compared to 10% and 15% extracts.

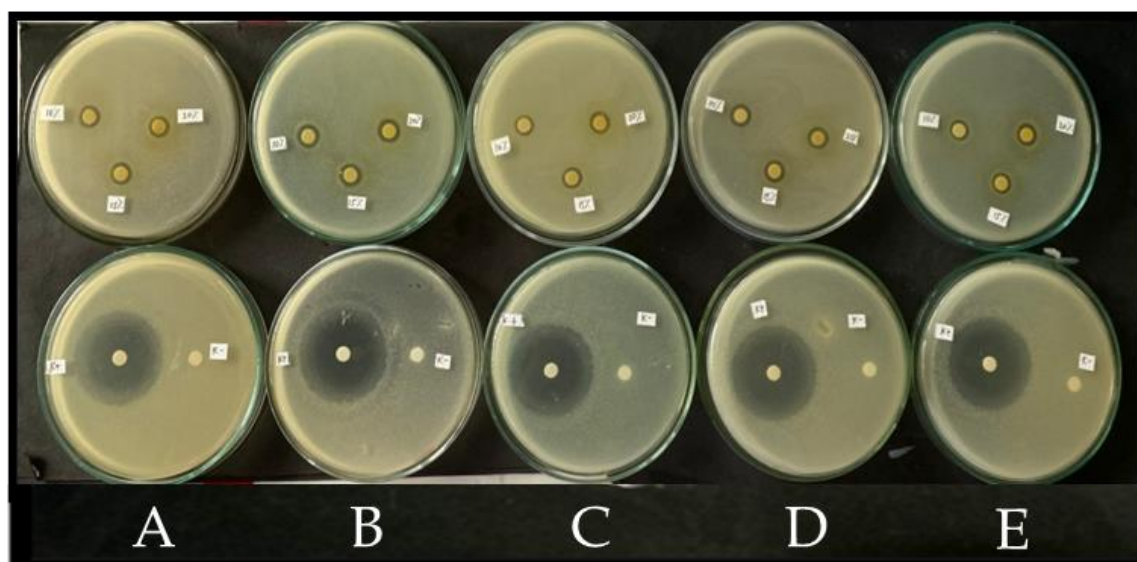


Figure 1. Formation of inhibition zones from turmeric leaf extract with concentrations of 10%, 15%, and 20% and positive control (clindamycin) on the growth of *S. sanguinis*. A: 1st repetition, B: 2nd repetition, C: 3rd repetition, D: 4th repetition, and E: 5th repetition.

According Febriani (2019), the criteria for the strength of antibacterial activity are as follows: an inhibition zone diameter of 5 mm or less is categorized as weak, an inhibition zone of 5-10 mm is classified as moderate, an inhibition zone of 10-20 mm is categorized as vital, and an inhibition zone of 20 mm or more is categorized as very strong.¹⁴ Strong. Extracts with 10% and 15% concentrations had antibacterial activity in the moderate category, while extracts with a concentration of 20% had antibacterial activity in the strong class. This proves that the higher the engagement, the larger the inhibition zone formed around the paper disc. The higher the concentration of an antibacterial substance, the stronger the antibacterial activity. This is in line with research conducted where the concentration of the substance influences the effectiveness of an antibacterial substance. Increasing the attention of a substance causes an increase in the content of active compounds that function as antibacterials so that the ability to kill bacteria is also greater.¹⁵

The ability of turmeric leaves to inhibit the growth of *S. sanguinis* bacteria is thought to be due to secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and triterpenoids. Alkaloids are polar compounds. In free form, alkaloids are weak bases that are difficult to dissolve in water but easily dissolve in organic solvents. The mechanism of action of alkaloids as an antibacterial is that the peptidoglycan component that makes up the bacterial cell is disrupted, resulting in lysis of the bacterial cell wall layer.¹⁶

Flavonoids are generally more soluble in water or polar solvents because they have bonds with hydroxyl groups. Flavonoids work as antibacterials with several mechanisms of action, including inhibiting the synthesis of nucleic acids, inhibiting the function of the cytoplasmic membrane, and inhibiting the energy metabolism of bacteria. Flavonoids work as antibacterials because of their ability to form complex compounds with extracellular and dissolved proteins that can damage the bacterial cell membrane and be followed by the release of intracellular compounds. Besides that, another mechanism of the flavonoid group in inhibiting bacterial growth is inhibiting the biofilm layer on bacteria.^{17, 18}

Saponins are generally glycosides, so they tend to be polar. Saponins are surface-active compounds that produce foam when shaken in water. This happens because saponins have polar and nonpolar groups that will form micelles. When the micelle is formed, the polar group will face out, and the nonpolar group will face inward to look like foam.¹⁹ The mechanism of saponins acts as an antibacterial by lowering surface tension resulting in increased permeability or cell leakage and causing intracellular compounds to come out. These compounds diffuse through the outer membrane and vulnerable cell walls and bind to the cytoplasmic membrane, disrupting and reducing stability.²⁰

Tannin, a phenolic compound, tends to dissolve in water and be polar. The mechanism of tannin as an antibacterial is related to inhibiting bacterial enzymes, where the transcriptase enzymes and DNA topoisomerase cannot be formed. In addition, tannins also have antibacterial activity

associated with inactivating microbial cell adhesin also, inactivating enzymes, and disrupting protein transport.²¹

Terpenoids are fat soluble. One of the terpenoids that have potential as an antimicrobial is triterpenoids, while steroids are a group of fats and are part of the triterpenoids. The mechanism of triterpenoids as antibacterials is reacting with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymer bonds that damage the porins.²² Damage to the porin, which is the entry and exit point for compounds, will reduce the permeability of the bacterial cell wall, resulting in a lack of nutrients in the bacterial cell, so that the growth of the bacteria is inhibited or dies.²³

The structure of the bacterial cell wall can determine the penetration of a substance, bonding, and activity of antibacterial compounds. *S. sanguinis* are gram-positive bacteria with a cell wall structure with more peptidoglycan, less lipids, and polysaccharides (teichoic acid).²⁴ Teichoic acid is a water-soluble polymer that is a favorable ion transport in and out of substances. This water-soluble nature indicates that the cell walls of gram-positive bacteria are more polar.²⁵ Turmeric leaves contain polar flavonoid compounds, so it is easier to penetrate the peptidoglycan layer in the bacterial cell wall. The incoming antibacterial compound will increase the cell's osmotic pressure, causing lysis.²⁶

This study's results align with previous research by Kasta (2020). The turmeric leaf extract contains active compounds of flavonoids, tannins, alkaloids, saponins, and triterpenoids with antibacterial properties.²⁷ Turmeric leaf extract was able to inhibit the growth of *Staphylococcus aureus* bacteria in the moderate category at a concentration of 6% (9.30 mm) and in the strong variety at a concentration of 12% (11.23 mm) and 18% (13.26 mm). This study also found that a turmeric leaf extract concentration of 20% effectively inhibited the growth of *S. sanguinis* because it produced an inhibition zone that was included in the strong category (10.18 mm). This study proves that turmeric leaf extract has an antibacterial effect on the growth of *S. sanguinis* in the oral cavity. This is confirmed by an inhibition zone (clear zone) that forms around the paper disc. This result is the first step in using turmeric leaves as an alternative antibacterial agent in dentistry.

4. Conclusion

Turmeric leaf extract (*Curcuma longa* L.) effectively inhibits the growth of *S. sanguinis* in vitro. At a concentration of 20% turmeric leaf extract (*Curcuma longa* L.) has the largest inhibition zone in inhibiting the growth of *S. sanguinis* in vitro with an average inhibition diameter of 10.18 mm.

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Authors Contribution

Contribution	Dohude G A	Rusdy H	Hanafiah O A	Br Ginting RAY
Concepts or ideas	V	V	V	V
Design		V	V	V
Definition of intellectual content	V			V
Literature search		V	V	V
Experimental studies	V		V	V
Data acquisition	V	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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