# Antibacterial Effect of Telang Flower (Clitoria Ternatea) Extract in Eradicating Streptoccus Mutans UA 159 Biofilm Mass

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**Abstract**

Antibacterial activity is indicated by the blue color on the telang flower (Clitoria ternatea) because it contains anthocyanins which are included in the flavonoid group. The purpose of this study was to examine the antibacterial activity of telang flower against Streptococcus mutans in eradicating S. mutans biofilm mass.

This research is a laboratory experimental study on the antibacterial activity of telang flower extract against Streptococcus mutans UA 159. The diameter of the inhibition zone was observed and the data obtained from measurement of the percentage of viability and inhibition were then statistically tested using the normality test, homogeneity test, and the Post Hoc Tukey HSD test. Further, calculate the eradication percentage of Streptococcus mutans biofilm formation by comparing the Optical Density (OD) value of each treatment.

There is antibacterial activity of the telang flower extract against S. mutans, with the value of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) in this study was at a concentration of 12.5 % and 100 % respectively. Telang flower extract was shown to significantly inhibit the growth of S. mutans ($p<0.05$) and with a concentration of 100% showed biofilm eradication results that were almost close to the positive control group.

Telang flower (Clitoria ternatea) extract has an antibacterial effect against and the ability to eradicate Streptococcus mutans biofilms. The level of inhibition shown by the telang flower extract on the growth of S. mutans was directly proportional to its concentration level, the higher the concentration of the extract, the greater the inhibition produced.

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## Introduction

Riset Kesehatan Dasar (RISKESDAS) in 2018 stated that 57.6% of Indonesian people had health problems related to dental and oral health. This can occur due to the lack of attention of the Indonesian people to dental and oral health, causing the incidence of dental and oral diseases to increase. If left untreated, this disease will cause pain, impair function, affect body weight, and disrupt growth and development which will ultimately affect the decrease in quality of life.[1-3]

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Dental and oral diseases are formed due to homeostatic imbalances in the oral cavity, such as changes in microorganisms that are influenced by salivary flow, salivary composition, sugar consumption, fluorine exposure, and also prevention factors. This condition is characterized by an increase in the number of Streptococcus mutans which will then colonize the tooth enamel surface.[4-8] These bacteria have the ability to synthesize extracellular polysaccharides, produce lactic acid, and form colonies that are firmly attached to the tooth surface, all of which contribute to the formation of biofilms.[9,10] The ability of S. mutans to adhere to tooth surfaces and form biofilms is one of its virulence factors.[11] This virulence factor also increases along with its ability to survive in an acidic environment.[10] The virulence factor of S. mutans is acid tolerance and production,

attachment of adesin to cell surface protein, production of glucosyltransferase (GTF), which is involved in the production of glucan, and intracellular polysaccharides. Furthermore, it will cause an increase in dental caries if left untreated due to the formation of a strong and cariogenic biofilm layer.[4-8]

It is well known that one of the etiological factors of dental caries is a microorganism, namely Streptococcus mutans in the oral cavity. One effort in the oral cavity to suppress the growth of bacteria is by using chemical agents in the form of mouthwash, such as chlorhexidine (CHX). Maintenance of dental and oral health can be done chemically by using mouthwash. However, continuous long-term use can irritation of the oral mucosa, disrupt the microbial balance in the mouth, unpleasant taste, causing staining of the teeth, and increasing bacterial resistance to antibacterials. As a result, efforts to locate safer, ideal, long-term-useable, and minimally harmful substitute materials remain necessary.[6,12]

Natural products are a new source of clinically applicable antibacterial agents. The antibacterial agents of plant extracts have been the subject of many reports. Polyphenols, such as phenolic acids, anthocyanins, flavonoids, coumarins, stilbenes, lignans, tannins and lignins, have high antibacterial activity due to their secondary metabolite content. Flowers, fruits, vegetables, and seeds contain a lot of substances that help build resistance to a variety of pathogenic microbes and protect against free radicals and toxins. [13-15]

Plants are a source of pharmacological compounds used as traditional medicine for several diseases such as microbial infections which are a significant public health problem and are always of interest to scientists. Recently many phytochemicals, including antibacterials, have been clarified from edible plants. Several studies have also been reported regarding herbal plant components, which have shown antibacterial activity against S. mutans.[16,17]

Recently, herbal plants are widely used as antibacterials because they have fewer side effects, inexpensive, and effective. One of them is telang flower (Clitoria ternatea), a plant that has long been used for treatment because of its active biological content, such as alkaloids, flavonoids, saponins, triterpenoids and steroids which have potential as antibacterials.[18,19] The content of flavonoids in this plant is known to have antibacterial properties due to the hydroxyl groups in the structure of flavonoids can cause toxic effects on bacteria because they cause changes in the transportation of organic components and nutrients.[20,21] Another component is alkaloids which can cause cell death by interfering with peptidoglycan in bacterial cells and preventing the formation of cell wall layers. Triterpenoids can bind to transmembrane proteins, causing damage to the bacterial cell membrane. It is the action of these components that causes the telang flower to be used as an antibacterial agent to prevent the occurrence of infectious diseases.[22,23]

Regular tooth brushing is one of the mechanical efforts in maintaining oral health which aims to eliminate biofilm but is less effective, therefore an effort is needed to use chemical agents in the form of mouthwash as an effort to prevent dental caries, so as to prevent bacterial growth in the oral cavity. Thus dental and oral health can be maintained by using mouthwash, but its use in the long term can cause side effects, so it is necessary to develop plants as natural ingredients containing biologically active components for oral prophylaxis.[6,24]

## Materials and methods

Laboratory experimental research on the antibacterial of telang flower (Clitoria ternatea) extract against Streptococcus mutans UA 159 with various concentrations on Mueller Hinton Agar (MHA) media was then carried out by observing the diameter of the inhibition zone and its ability to eradicate S. mutans biofilm mass.

The first step is the preparation of growing media using Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB). Thirty eight grams of MHA media and 21 grams of MHB media were weighed using an alytical balance. Both were then dissolved respectively in 1000 ml of ddH2O with the help of a microwave. After that, it was sterilized for 20 minutes in an autoclave at 121°C and 1.5 atm pressure.

Telang flower (Clitoria ternatea) was used in this study. Furthermore, the preparation of telang flower extract was carried out by preparing a stock solution, making a series of Working Solution (WS), and sample filtration. The telang flower extract stock solution used was prepared

by dissolving 2 mg of extract paste in 1 ml of 100% DMSO so that the stock solution has a concentration of 2 mg/ml of extract in 100% DMSO.

The Working Solution (WS) series was made by diluting the stock of telang flower extract using ddH2O then using 10% DMSO to make the concentration series. The extract concentration series used are:

TFE 100%: 50 µl stock solution + 950 µlddH2O (1st solution)

TFE 50%: 500 µl 1st solution + 500 µl DMSO 10% (2nd solution)

TFE 25%: 500 µl 2nd solution + 500 µl DMSO 10% (3rd solution)

TFE 12.5%: 500 µl 3rd solution + 500 µl DMSO 10% (4th solution)

TFE 6.25%: 500 µl 4th solution + 500 µl DMSO 10% (5th solution)

TFE 3.125%: 500 µl 5th solution + 500 µl DMSO 10%

The working solution was then filtered using a 0.22 um pore filter syringe to obtain a sterile sample.

The direct colony suspension method was used to make S. mutans inoculums. Inoculums were obtained by inoculating S. mutans colonies on MHA medium into MHB. To obtain an inoculum with a bacterial count of around 1-5×$10^8$ CFU/ml, the turbidity of the solution was then adjusted visually to the turbidity of 0.5 McFarland standard solution. Then the solution was diluted using MHB to produce inoculums with bacterial counts in the range 2×$10^6$ - 1×$10^7$CFU/ml with a ratio of 1:50. Then dilution was carried out using MHB media with a ratio of 1:20 to produce an inoculum with a bacterial count of 1-5×$10^5$ CFU/ml.

The broth microdilution method was carried out in wells by adding 100 µl of inoculum of various concentrations of telang flower extract, then adding 100 µl of the working solution for each concentration of the extract in the well so that the extract concentration reached the final concentration and 100 µl of chlorhexidine 0.2% was added to the well. Furthermore, for growth control, 100 µl MHB and 100 µl inoculum were added to the well. As a blank, add 100 µl of MHB and 100 µl of working solution to the well for each concentration of telang extract, then also make a 0.2% Chlorhexidine blank. Incubate the plate for 24 hours in an incubator at 37°C, followed by measurements using spectrophotometry with a wavelength of 530 nm. The Optical Density (OD) values of the treatments and each blank were compared to determine the growth of S. mutans. The lowest extract concentration with a 99 percent inhibition of bacterial growth was used to calculate the MIC value.

The pour plate method was carried out after spectrophotometric measurements of each treatment in the microplate. This method is carried out to prove that the minimum concentration of bactericidal observed in spectrophotometric measurements is able to provide an inhibitory effect so that there is no growth of bacterial colonies on the agar medium. Then carry out the pour plate method by taking 20 µl of solution from each well and growing it on MHA media, then incubating at 37°C for 24 hours. Visual observations were made then the number of colonies of bacteria was counted using a colony counter. The MIC value was proven by the concentration of the extract which was able to inhibit bacterial growth so that no growth of bacterial colonies was observed on the agar medium.

The data obtained from the measurement of the percentage of viability and inhibition were statistically tested. In this study, the statistical tests used were the normality test, homogeneity test, and the Post Hoc Tukey HSD test. The IBM Statistics SPSS 25 application is used to perform statistical tests.

The Streptococcus mutans biofilm eradication test was then carried out by culturing Streptococcus mutans on Brain Heart Infusion Medium at 37°C for 24 hours until the bacteria were at 0.5 Mc Farland turbidity. As much as 50 µL of Streptococcus mutans was put into 96 well plates then added 100 µL of telang flower extract with each concentration into 96 well plates which already contained the bacterial culture. Incubation for 48 hours at 37°C. After incubation was complete, the microplate incubation liquid was removed and then washed with PBS solution 2 times. After that, 125 µL of Crystal Violet 0.1% was added to the well and incubated for 10-15 minutes, the microplate was rinsed using distilled water 3-4 times. Then 125 µL of 30% acetic acid was added to the well plate which had been stained with 0.1% Crystal Violet. Then measured with a microplate reader with a wavelength of 490 nm. By comparing the OD values of each treatment and each blank, biofilm growth was

identified.

The formula used to calculate the eradication percentage for Streptococcus mutans biofilm formation is:

$$\%\ inhibition = \left[1 - \frac{OD\ sample - OD\ sample\ blank}{(OD\ solvent - OD\ solvent\ blank)}\right] x\ 100\%$$

Information:

OD sample : Optical density of telang flower extract + solvent + bacterial suspension.

OD sample blank: Optical density of telang flower extract + solvent.

OD solvent : Optical density control of solvent + bacterial suspension.

OD solvent blank: Optical density control of solvent.

The examination was carried out 3 times and the average OD value was determined. Biofilm formation is measured by:

Biofilm formation = OD at 490nm in bacterial assay – OD control

## Results

The results of this study indicate that there is an antibacterial activity of telang flower (C. ternatea) extract against S. mutans. In this study, the MIC and MBC of telang flower extract against S. mutans was 12.5% and 100%, perspectively, as can be seen in Table 1. The value of the MBC of telang flower extract against S. mutans in this study was 100% as shown in Table 1.

| Sample | Viability (%) | | | Inhibition (%) | | | |
|---|---|---|---|---|---|---|---|
| **TFE 100%** | **1.35** | **±** | **0.10$^{a}$** | **98.65** | **±** | **0.10$^{g}$** | **MIC** |
| TFE 50% | 8.44 | ± | 0.36$^{b}$ | 91.56 | ± | 0.36$^{f}$ | |
| TFE 25% | 15.75 | ± | 0.43$^{c}$ | 84.25 | ± | 0.43$^{e}$ | |
| **TFE 12.5%** | **41.67** | **±** | **0.60$^{d}$** | **58.33** | **±** | **0.60$^{d}$** | **MBC** |
| TFE 6.25% | 90.47 | ± | 0.59$^{e}$ | 9.53 | ± | 0.59$^{c}$ | |
| TFE 3.125% | 97.47 | ± | 0.24$^{f}$ | 2.53 | ± | 0.24$^{b}$ | |
| GC (Growth Control) | 100.00 | ± | 0.19$^{g}$ | 0.00 | ± | 0.19$^{a}$ | |
| Chlorhexidine 0.2% | 1.25 | ± | 0.19$^{a}$ | 98.75 | ± | 0.19$^{g}$ | |

**Table 1.** Viability and Inhibition of Telang Flower Extract (TFE) against S. mutans.

*Data presented is the average ± standard deviation. Letters (a,b,c,d,e,f,g) indicate a significant difference based on the Tukey HSD test (p<0.05).

The extract of the telang flower (C. ternatea) was shown to inhibit the growth of S. mutans significantly (p<0.05) as shown in Figures 1 and 2. The inhibition ability of telang flower extract on the growth of S. mutans bacteria can be said to be directly proportional to its concentration, the inhibitory effect will be higher if the concentration of the extract is also higher.

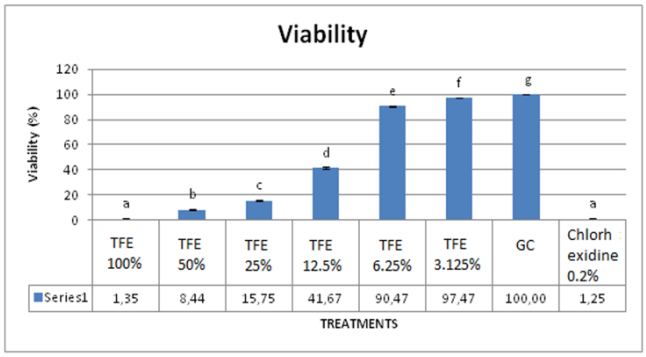


**Figure 1.** Graph of Viability Percentage of S. mutans after administration of Telang Flower Extract.

*Data presented is the average ± standard deviation. Letters (a,b,c,d,e,f,g) indicate a significant difference based on the Tukey HSD test (p<0.05).

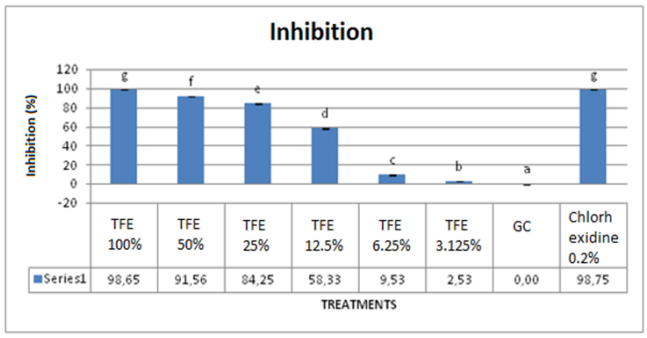


**Figure 2.** Percentage of Inhibition of Telang Flower Extract against S. mutans.

*Data presented is the average ± standard deviation. Letters (a,b,c,d,e,f,g) indicate a significant difference based on the Tukey HSD test (p<0.05).

| Sample | Dilution factor | Number of colonies | | | CFU/ml | | | Average | Average |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 1 | 2 | 3 | | |
| PC | 10000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GC | 10000 | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC | TNTC |
| TFE 100% | 10000 | 3 | 0 | 0 | 0 | 3 x $10^{4}$ | 0 | 1.00 | 1 x $10^{4}$ |
| TFE 50% | 10000 | 25 | 20 | 29 | 25 x $10^{4}$ | 20 x $10^{4}$ | 29 x $10^{4}$ | 24.67 | 24.67x$10^{4}$ |
| TFE 25% | 10000 | 40 | 35 | 37 | 40 x $10^{4}$ | 35 x $10^{4}$ | 37 x $10^{4}$ | 37.33 | 37.33x$10^{4}$ |
| TFE 12.5% | 10000 | 79 | 78 | 82 | 79 x $10^{4}$ | 78 x $10^{4}$ | 82 x $10^{4}$ | 79.67 | 79.67x$10^{4}$ |
| TFE 6.125% | 10000 | 147 | 151 | 149 | 147 x $10^{4}$ | 151 x $10^{4}$ | 149 x 10 | 149.00 | 149x$10^{4}$ |
| TFE 3.125% | 10000 | 190 | 198 | 199 | 190 x $10^{4}$ | 198 x $10^{4}$ | 199 x 10 | 195.67 | 195.67x$10^{4}$ |

**Table 2**. Calculation of the number of colonies of telang flower extract in the MIC and MBC tests.

TNTC : *Too Numerous To Count*

PC (Positif Control):S. mutans inoculum + Chlorhexidine 0.2%

GC (Growth Control): S. mutans inoculum + MHB (Mueller Hinton Broth)

Streptococcus mutans biofilm eradication testing showed that all treatment groups were able to eradicate biofilms with the highest inhibition percentage being the telang flower extract treatment group with a concentration of 100%. While the treatment group that showed

the lowest results in eradicating S. mutans biofilm was the telang flower extract treatment group with a concentration of 3.125%. (Table 3)

| Group | Inhibition (%) | | | Average |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Negative control | 3.36 | 3.33 | 3.28 | 3.33 |
| TFE 3.125% | 4.27 | 4.18 | 4.10 | 4.19 |
| TFE 6.25% | 12.26 | 12.25 | 12.16 | 12.23 |
| TFE 12.5% | 53.75 | 53.72 | 52.46 | 53.31 |
| TFE 25% | 88.02 | 87.92 | 87.93 | 87.96 |
| TFE 50% | 94.64 | 94.60 | 94.59 | 94.61 |
| TFE 100% | 99.96 | 99.98 | 99.93 | 99.96 |
| Positive control | 100.00 | 100.00 | 100.00 | 100.00 |

**Table 3.** Results of Biofilm Eradication Test of Telang Flower Extract.

## Discussion

The Clitoria ternatea plant is widely used as a traditional herbal medicine in Indonesia. Although all parts of the plant can be used, the flower parts are most often used due to their antimicrobial, anti-inflammatory, and antioxidant properties. The antibacterial potential of various parts of the C. ternatea plant in maintaining oral health is not known with certainty, therefore this study was conducted to determine the antibacterial activity of telang flower extract on the growth of Streptococcus mutans bacteria because this bacterium plays a very important role in the occurrence of dental caries. [5-8]

Herbal plants such as telang flower used alone or in combination have been scientifically proven to be safe and effective drugs without side effects. The main advantage of using herbal plants is the minimal side effects and so far there have been no reports of any side effects.[25] Telang flower extract has an antibacterial activity which results in a decrease in the number of bacteria so that the ability of bacteria to colonize and communicate with each other is hampered.[26,27]

Dental caries is thought to be caused by Streptococcus mutans, which can form biofilms on the surface of teeth. Its capacity to obtain nutrients and metabolize fermentable dietary carbohydrates into acids contributes to its pathogenicity. The response of S. mutans to environmental stress is critical for its virulence and survival. The VicRK system is one of the two-component signal transduction systems (TCS) that is thought to exist in S. mutans. The function of the VicRK signal transduction system is the main regulatory factor for acidification, cell wall metabolism, biofilm formation, and bacterial oxidative stress response so that it can cause S. mutans to survive.[28,29]

Chlorhexidine is a gold standard antimicrobial that acts as a very broad spectrum antimicrobial, considered as an agent for evaluating the effectiveness of other antimicrobials, antiplaques, and herbal extracts, so this study was used as a positive control. Chlorhexidine is considered one of the best antimicrobial agents in controlling the growth of oral bacteria.[30-35] Chlorhexidine, like other drugs, is certainly not without side effects. Chlorhexidine and herbs are both effective for biofilm control in preventing plaque and gingivitis. However, due to the reported side effects due to the use of chlorhexidine as well as the good biocompatibility and acceptability of herbs, herbs can be used effectively as alternatives to chlorhexidine.[36]

In this study, the percentage inhibition of S. mutans flower extract against S. mutans at a concentration of 100% was as good as 0.2% Chlorhexidine (Tables 1 and 2). Based on table 3, telang flower extract can eradicate Streptococcus mutans biofilms. The telang extract treatment group with a concentration of 100% showed the highest results among other concentrations with an average of 99.96% and almost close to the positive control group which had an average value of 100.00%. While the telang extract treatment group with a concentration of 3.125% showed the lowest results compared to other concentrations, namely an average of 4.19%. This is because the highest content of telang flower extract is flavonoids, saponins, terpenoids, and alkaloids.[19,21,31,37-39]

Flavonoids can inhibit the function of cell membranes and penetrate the bacterial lipid bilayer, thus damaging the outer membrane barrier function. Furthermore, these compounds cause membrane fusion and result in cell leakage. However, this compound was less successful at penetrating the lipopolysaccharide membrane of gram-negative bacteria. Therefore, the antibacterial mechanism is limited. In another study, flavonoids inhibited bacterial energy metabolism which is necessary for the synthesis of macromolecules (DNA, RNA, and proteins).[20,31]

The role of saponins as an antibacterial is by denaturing proteins by reducing the surface tension of the bacterial cell wall which causes

lysis, damage or disturbance to the permeability of the bacterial cell membrane.[40,41] In addition to saponins, terpenoids also have antibacterial activity by destroying transmembrane proteins through their reactions with transmembrane proteins on the outer membrane of bacterial cells and forming strong polymeric bonds.[42,43]

Terpenoids are carbon dioxide, meaning they contain the elements carbon and hydrogen which have been denatured by oxidation or chemically modified. Terpenoids represent a large class of antibacterial compounds produced by plants. Terpenoids originate from five-carbon isoprene units and most of the terpenoids have multicyclic structures that differ from one another in their functional groups and their basic carbon skeleton.[42-44]

The structure of other compounds, namely alkaloids which are derivatives of furoquinoline, these compounds can cause interference with the formation of the bacterial cell wall layer by interfering with the constituent components of peptidoglycan will cause cell death and depolarizing the cell wall.[22,45,46] In this plant tannins have phenol groups, these components can form complex compounds, which cause bacterial cells to die due to the incomplete formation of bacterial cell walls.[47,48]

The ability of telang flower extract to react with cell membrane activity, energy production, the synthesis of structural components, and disrupting the function of genetic material, can cause disruption of the activity of the glucosyltransferase enzyme used by S. mutans to synthesize sucrose in glucan medium which is a bacterial attachment medium. If the amount of glucan is small, it will inhibit the formation of biofilms.[49-51]

## Conclusions

Telang flower (Clitoria ternatea) extract has an antibacterial effect against and the ability to eradicate Streptococcus mutans biofilms. The level of inhibition shown by the telang flower extract on the growth of S. mutans was directly proportional to its concentration level, the higher the concentration of the extract, the greater the inhibition produced.

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## Declaration of Interest

The authors declare no conflict of interest.

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