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STRAWBERRY (*Fragaria × ananassa*) FRUIT EXTRACT AS ANTIBACTERIAL AGAINST THE GROWTH OF *Streptococcus sanguinis*

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ABSTRACT

Background: Strawberry (*Fragaria × ananassa*) is a natural substance known to reduce dental plaque formation due to its content of bioactive compounds, including saponins, alkaloids, tannins, triterpenoids, and steroids. The **purpose of this study was to** assess strawberry fruit extract's antibacterial properties, prepared using 96% ethanol as a solvent through maceration to produce a concentrated liquid extract, against *Streptococcus sanguinis*—a bacterium associated with plaque formation.
Method: The study employed microdilution and agar dilution methods. Antibacterial activity was assessed through the Minimum Inhibitory Concentration (MIC), indicated by a marked decrease in optical density, and the Minimum Bactericidal Concentration (MBC), determined by the absence of bacterial colony growth.
Outcome: According to the results, the strawberry extract's MBC was 50%, and its MIC was 6.25% against Streptococcus sanguinis.
Conclusion: These findings indicate that strawberry extract (*Fragaria × ananassa*) exhibits both inhibitory and bactericidal activity against the growth of *Streptococcus sanguinis*.

INTRODUCTION

The oral cavity, one of the body's most highly colonized areas, is perfect for microbiome inhabitants. Around 700 different microbiota species are estimated to live in the oral cavity.[1] Dental and oral hygiene are important to maintain the balance of body functions and are one of the determining factors for dental and oral health.[2] Recent studies indicate that dental and oral health remains a significant concern in Indonesia, with a high prevalence of oral health problems.[3] Based on data from Survei Kesehatan Indonesia (SKI), the prevalence of dental and oral health problems in the Indonesian population was 56.9%.[4] Regular oral hygiene is crucial for preventing dental diseases and maintaining overall health. Plaque accumulation from poor oral hygiene is linked to a higher risk of periodontal disease and dental cavity.[5]

The breakdown of the periodontal complex's (including soft and hard tissues in periodontal complexes), caused by microbial communities and unusual immune responses, is a hallmark of periodontal disease.[6] The primary etiological agent for periodontal disease is dental plaque biofilm.[7] Dental plaque formation is primarily bacteria such as *Streptococcus mutans, Streptococcus salivarius*,

and *Streptococcus sanguinis* which colonize tooth surfaces and produce biofilms.[8,9] *Streptococcus sanguinis* plays a significant role in dental plaque formation that produces glucosyltransferases that synthesize glucan from dietary sucrose, contributing to biofilm development.[9]

Efforts to prevent this are by using antibacterial mouthwash. Chlorhexidine is a non-herbal mouthwash that has long been known as a plaque control agent.[10] The antiplaque effect found in chlorhexidine has good bactericidal and bacteriostatic properties.[11] The use of chlorhexidine cannot be used in the long term because it has undesirable side effects such as changes in taste in the oral cavity and extrinsic tooth staining on enamel cracks.[10] Strawberries are one of the herbal alternatives to chlorhexidine, which has side effects. Strawberries, which have the Latin name *Fragaria x ananassa*, are not only popular for their taste and aroma but also recognized as a functional food with significant health benefits.[12] Strawberries contain various polyphenolic compounds, such as tannins and flavonoids.[13] Tannins and flavonoids have antibacterial properties. Flavonoids can interrupt the integrity of bacterial cell walls by forming complexes with extracellular proteins, while tannins can shrink these cell walls, thereby inhibiting microbial extracellular enzymes and oxidative phosphorylation.[14] Based on those, we're interested on the antibacterial effects and determining the inhibitory and killing power of strawberries to prevent plaque growth.

RESEARCH METHODS

Study Design

Experimental laboratory study is used for this research using the maceration method to determine the antibacterial activity effect of strawberry extract (*Fragaria x ananassa*) against *Streptococcus sanguinis*, measuring minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using microdilution technique and agar dilution technique. This research is conducted in the Microbiology Center of Research and Education (MiCore) laboratory, Jakarta.

Bacterial Strain

The sample of this study used *Streptococcus sanguinis* ATCC 10556 bacteria.

Sample Collection and Preparation

The strawberries used in this study were taken from plantations in Lembang, West Java and were tested for determination at the Padjajaran University Laboratory, Bandung.

Preparation of Ethanolic Strawberry Extract

Strawberries are separated from whole plants and weighed using a balance scale, then dried under the sun and reweighed as much as 2000 grams. Dried strawberries are put into a maceration vessel and soaked with 96% ethanol for 24 hours and sometimes stirred. This maceration is repeated several times.

The soaking results are filtered with filter paper. The filtrate is then collected in a container. To obtain ethanol and strawberry extract, the filtrate is concentrated at 50°C in a vacuum rotary evaporator and then evaporated in a water bath. Next, the extract is put into a container and stored in the refrigerator.

Phytochemical Test of Strawberry Fruit Extract

Strawberry fruit extract was subjected to phytochemical tests to see and determine the content of active compounds contained in the plant. Some phytochemical tests carried out were saponin, flavonoid, tannin, triterpenoid, alkaloid, and steroid tests. Alkaloids were tested using 3 ml of strawberry fruit extract added with a few drops of H_2SO_4 in a test tube, shaken until separated into 2 layers, and then filtered. Then 1 ml extract from the test tube was dripped with two drops of Mayer's reagent. Orange to reddish-brown precipitates indicated alkaloid compounds. To test flavonoids, 3 ml of strawberry fruit extract was added to 10 ml of hot water at a temperature of 70℃. Magnesium powder and concentrated HCl were added to the test tube and then shaken. The formation of red, orange, or purple indicates positive flavonoids. Saponins were tested using 3 ml of strawberry fruit extract added to 10 ml water at 70℃, and the test tube was shaken vigorously to check the foam formation for 10 minutes. If there is foam with a height of 1-10 cm that lasts for 10 minutes, then the extract is positive for saponins. Tannins were tested by adding a few drops of 1% $FeCl_3$. A positive test is indicated by the formation of dark black, green, blue, purple, or red. Lastly, the steroids and triterpenoids were tested using 2 ml of strawberry fruit extract added to Lieberman and $CHCl_3$ reagents. Steroids give a blue or green color, and triterpenoids showed a red or purple color.

Preparation of *Streptococcus sanguinis* Suspension

Dissolve **10.5 grams of Mueller Hinton Broth (MHB) in 500 ml double-distilled water (ddH2O)**, then heat it to boiling and fully mixed. Following that, they were autoclaved for 20 minutes at 121 °C to sterilize. MHB is inoculated with *Streptococcus sanguinis* colonies that have been grown on Muller Hinton Agar (MHA) medium. The suspension is homogenized with a vortex mixer. An inoculum with a bacterial count of approximately 1.5 × 108 CFU / ml (colony forming unit) / ml is then obtained by adjusting the turbidity to the McFarland 0.5 standard solution.

Bacterial Culture Preparation

After **dissolving 19 grams of MHA medium in 500 mL** of **ddH_2O**, the mixture is heated until boiling and homogeneous. After that, the medium was sterilized using an autoclave for 20 minutes at 121℃. The medium was **poured into** sterile petri dishes to form agar plates. After adjusting the turbidity of the bacterial suspension with a McFarland 0.5 standard solution, a sterile cotton swab was used to dip into it and evenly swabbed over the surface of the agar plates. The plates were left for 3–5 minutes at room temperature to allow absorption before testing.

Minimum Inhibitory Concentration (MIC) Determination

This research used the microdilution method. 100 µL of 0.2% chlorhexidine (positive control) and 100 µL of 10% DMSO (negative control) were inserted into wells 1A-1C and 2A-2C. Then 100 µL of MHB was inserted into wells 3A-7A, 3B-7B, and 3C-7C. 200 µL of strawberry fruit extract (*Fragaria x ananassa*) was inserted into wells 8A-8C. 100 µL of the solution in well 8A-8C was taken and then inserted into well 7A-7C and the solution was homogenized using a micropipette. This step is repeated until well 3A-3C. 100 µL of solution in wells 3A-3C is discarded. 100 µL of bacterial suspension was added to the 24 wells microdilution plate and homogenized with a micropipette. Then the plate was covered and incubated. Incubation at 37°C for 24 hours. The wells are observed using a spectrophotometer with a wavelength of 600nm. The minimum inhibitory concentration was based on the lowest concentration that could inhibit bacterial growth as indicated by the clarity of the media using the difference in optical density (ΔOD) before and after incubation.

Bacterial Growth Curve and Cell Count Estimation

The bacterial standard curve is used to calculate the number of bacteria by regressing the absorbance value and the number of colonies into the standard curve line equation with the formula $y = ax + b$, where y is the number of colonies and x is the absorbance value. The method to calculate the number of *Streptococcus sanguinis* bacterial cells used to create the standard curve of this study involves using a spectrophotometer to measure the turbidity level through the absorbance value produced. The linear regression equation will be obtained using a graph to determine the number of bacterial cells on the bacterial growth curve.

Minimum Bactericidal Concentration (MBC) Determination

The microdilution results were put into a petri dish containing Mueller Hinton agar (MHA) and leveled with a spreader. The petri dish was put into an anaerobic jar under anaerobic conditions and incubated using an incubator for 24 hours at 37°C. *Streptococcus sanguinis* colonies were observed by counting the number. MBC is the smallest concentration where no bacterial colonies grow from strawberry fruit extract (*Fragaria x ananassa*).

Statistical Analysis

Shapiro-Wilk is used to test data normality and Levene is used to test data homogeneity. Then a test will be carried out using Kruskal-Wallis and Pairwise Comparison data analysis methods. A p-value < 0.05 was considered statistically significant.

RESEARCH FINDINGS

Plant Identification

The plant used in this study was identified to ensure the authenticity of the sample. The results of the identification are as follows: scientific name: *Fragaria x ananassa* (Duchesne ex Weston), synonym: *Potentilla x ananassa*, local name: Stroberi (Strawberry), family: Rosaceae Juss.

Phytochemical Screening

Secondary metabolite compounds such as phenolics, tannins, saponins, tripernoids, and alkaloids were found. These results are summarized in Table 1.

Table 1. Phytochemical Screening Results

No	Secondary Metabolite	Result
1	Phenolics	+
2	Tannins	+
3	Flavonoids	-
4	Saponins	+
5	Triterpenoids	+
6	Steroids	-
7	Alkaloids	+

Minimum Inhibitory Concentration (MIC) Test

Table 2 showing the results of the procedure. The first decrease in the ΔOD value was obtained at a concentration of 6.25%. indicating the MIC.

Table 2. MIC Test Results of Strawberry (*Fragaria x ananassa*) Fruit Extract

Treatment Group	ΔOD (Before – After Incubation)
Negative Control	0,272
1.5625% Extract	0,397
3.125% Extract	0,383
6.25% Extract	-0,015 (MIC)
12.5% Extract	-0,031
25% Extract	-0,193
50% Extract	-0,193
100% Extract	-0,126
Positive Control	0,603

Bacterial Growth Curve and Cell Count Estimation

Table 3 will be used to create a standard curve, that will show the relationship between absorbance on the y-axis and the log number of bacterial cells on the x-axis.

Table 3. Standard Growth Curve Data

Treatment Group	Absorbance	
	Before Incubation	After Incubation
Negative Control	0.111	0.383
1.5625% Extract	0.349	0.746
3.125% Extract	0.673	1.056
6.25% Extract	1.257	1.242
12.5% Extract	1.998	1.967
25% Extract	2.963	2.770
50% Extract	3.160	3.147
100% Extract	3.551	3.425
Positive Control	0.133	0.736

The following are the results of the average log before incubation and after incubation by entering the linear equation line as shown in Figure 1 with y = 311.32x – 120.02 and Figure 2 with y = 266.42x – 179.81. These equations were used to calculate the logarithmic values of bacterial cell counts before and after incubation presented in table 4. These data further support the antibacterial effect of strawberry extract, as indicated by a decrease in bacterial growth at specific concentrations.

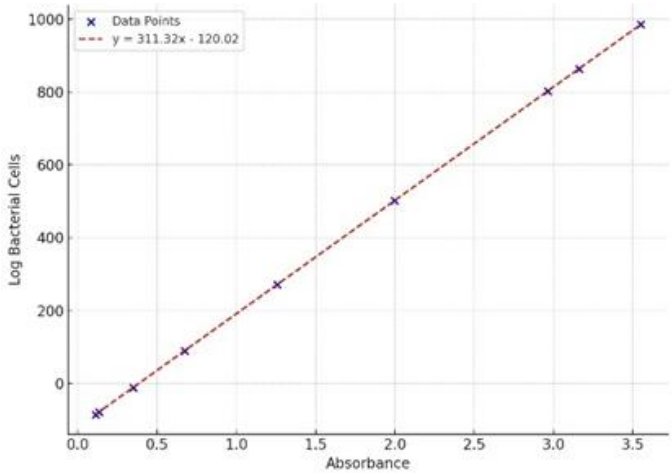

Figure 1. Bacterial growth curve before incubation

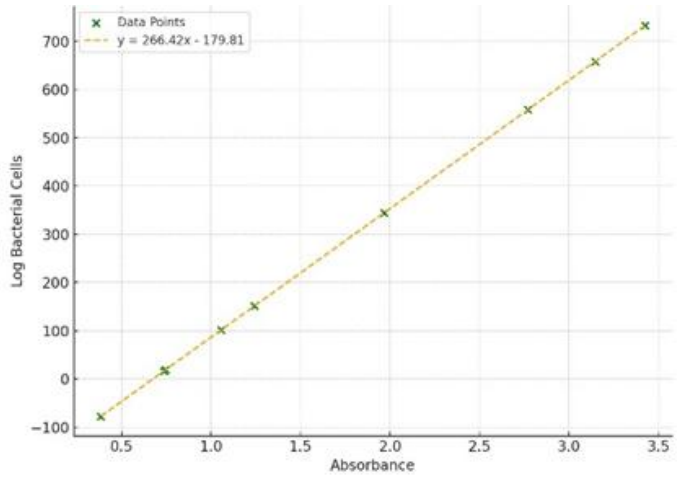

Figure 2. Bacterial growth curve after incubation

Table 4. Logarithmic Cell Counts Before and After Incubation

Treatment Group	Log Cell Count	
	Before Incubation	After Incubation
Negative Control	–85.47	–77.77
1.5625% Extract	–11.38	18.94
3.125% Extract	89.49	101.53
6.25% Extract	271.30	151.08
12.5% Extract	501.99	344.24
25% Extract	802.41	558.17
50% Extract	863.74	658.61
100% Extract	985.47	732.68
Positive Control	–78.62	16.28

Minimum Bactericidal Concentration (MBC)

The test results can be seen in Table 5. Based on this result, the MBC was at a concentration of 50% with an average of 0 bacterial colonies. A slight bacterial presence was still noted at 100% and 25%, indicating strong but slightly variable bactericidal effects. Concentrations lower than 25% resulted in increasing bacterial growth, with the highest colony counts observed at 3.125% and 1.56% concentrations. The negative control showed extensive growth, while the positive control limited bacterial presence to 7.3 colonies. These findings demonstrate that the bactericidal effect of the strawberry fruit extract becomes significant at a concentration of 50%, where complete bacterial elimination was achieved.

Table 5. Minimum Bactericidal Concentration (MBC) Test Results of Strawberry (*Fragaria x ananassa*) Fruit Extract

Treatment Group	Colony count (mean)
Negative Control	601,3
1.5625% Extract	579,6
3.125% Extract	623,3
6.25% Extract	42,6
12.5% Extract	7,6
25% Extract	1,3
50% Extract	0 (MBC)
100% Extract	1,6
Positive Control	7,3

Statistical Analysis

The MIC and MBC data were analyzed. Before analyzing the research data, a normality test and a homogeneity test were first conducted. The data normality test using Shapiro-Wilk showed p-value < 0.05. The homogeneity test used was Levene's, and it was found that both groups had a p-value < 0.05. This showed that both had non-normal distributions and were not homogeneous. Data analysis from the MIC and MBC procedures used is Kruskal-Wallis, and it was found that the MIC and MBC procedures have p-values < 0.05, indicating that there is a significant difference in the test group for both methods.

DISCUSSION

According to the results of the phytochemical tests that have been carried out, strawberry fruit extract (*Fragaria x ananassa*) has proven to have secondary metabolite compounds. The secondary metabolite that found in this research are phenolics, tannins, alkaloids, triterpenoids, and saponins. However, the results in this study showed differences from previous studies, where no flavonoid and steroid compounds were found in strawberry fruit extract (*Fragaria x ananassa*). This can be caused by several factors, such as inadequate solvent polarity or improper extraction conditions (temperature, time, or solvent/solid ratio), over ripeness or improper storage, or degradation during processing.[15–18]

Based on the results of the study, strawberries (*Fragaria x ananassa*) can inhibit growth and kill *Streptococcus sanguinis* bacteria. The determination of the MIC is done by comparing optical density before and after incubation. The inhibitory concentration value is obtained if the optical density ≤ 0. A negative ΔOD value indicates a decrease in absorbance, which means that the number of bacteria decreases after 24 hours of incubation.[19] Standard curve data and log cell calculation results also show that the extract has an inhibitory effect, which is indicated by a decrease in bacterial viability. The MBC was obtained at a concentration of 50%. A concentration of 50% is the minimum concentration that can stop the growth of *Streptococcus sanguinis*, where no bacterial colony growth was found with a colony count of 0. Strawberry fruit extract (*Fragaria x ananassa*) can inhibit and stop the growth of bacterial colonies of *Streptococcus sanguinis* due to the mechanism of several metabolite compounds contained in the extract used.[20]

The results of the agar dilution procedure with a concentration of 100% strawberry extract had an average of 1.6 bacterial colonies. This can occur due to contamination of the culture medium. Contamination of the culture medium can occur due to internal and external factors such as the entry of microorganisms into the medium, dirty work and culture rooms, less sterile planting tools, and negligence in implementation. Sterilization is the main factor that determines the success of media culture.[21,22]

The effectiveness of strawberry fruit extract (*Fragaria x ananassa*) to inhibit and kill *Streptococcus sanguinis* is related to the presence of antibacterial compounds in it.[23] These metabolite compounds have an antibacterial effect. The content of strawberry fruit extract (*Fragaria x ananassa*) that can inhibit and kill bacteria include saponins, alkaloids, phenolic compounds, tannins, triterpenoids, and flavonoid. Each compound has a different mechanism of action.[24,25] The nature of the active substance saponin through their surfactant-like properties allows saponins to act as antibacterials. This is caused by a decrease in surface tension on **the bacterial cell wall** and damage to **the permeability** of the bacterial membrane causing membrane lysis and leakage of intracellular contents.[26–28]

Antibacterial mechanism of action of alkaloids is by inhibiting the formation of cross-bridges between peptidoglycan components in bacterial cells. This causes the cell wall to not form perfectly and interfering with cell wall integrity and enzyme function of cell proteins which causes bacteria exposed to the compound to experience inhibited growth and induce cell death.[29,30]

Phenolic compounds have the potential as antibacterial agents by acting primarily by increasing membrane permeability and promoting oxidative stress in bacterial cells that damage the protein structure in the bacterial cell membrane so that growth is inhibited and eventually dies.[31,32] Phenolic compounds are compounds that have hydroxyl groups bound to aromatic rings and have been widely studied as disinfectants that have antibacterial activity covering various types of bacteria.[33]

Tannins, plant-derived polyphenolic compounds, exhibit potent antibacterial properties against various pathogens. These compounds act as broad-spectrum antimicrobial agents by inhibiting enzymatic activities and nucleic acid synthesis.[34] Tannins inhibit reverse transcriptase and DNA topoisomerase enzymes that prevent the formation of bacterial cells. In addition, function by binding to bacterial surface proteins and polysaccharides, destabilizing membrane integrity and disrupting metabolic activity. This causes bacterial cells to lysis due to osmotic pressure.[35–38]

Triterpenoids are included in the content of compounds that have antibacterial activity by interacting with bacterial porins in the outer membrane, hindering essential molecule transport and energy transduction of microorganisms such as bacteria.[39,40] Triterpenoids and flavonoids also show synergistic antibacterial activity against Gram-positive and Gram-negative bacteria.[41]

Flavonoids, which are typically found in strawberries, were not found in this study. Flavonoids are known to have the ability to inhibit the synthesis of nucleic acid and damage the cytoplasmic membrane that act as antibacterial activity for bacteria.[42] The absence of flavonoids in this study could be caused by factors such as plant collection methods, storage of plant materials, lighting, or testing processes. Therefore, attention is needed in plant collection techniques and storage of plant materials so that active compounds are not damaged. Environmental conditions, such as temperature, can also impact flavonoid accumulation.[43,44]

CONCLUSION

Ethanol extract of strawberry fruit (*Fragaria x ananassa*) has the ability **to inhibit the growth** and kill *Streptococcus sanguinis* with a minimum inhibitory concentration (MIC) of 6.25% and a minimum bactericidal concentration (MBC) of 50%. The antimicrobial effect of this extract is likely to come from the combination of phenolics, tannins, alkaloids, triterpenoids, and saponins, and can be used as an antibacterial, especially to treat *Streptococcus sanguinis* bacteria.

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