The Effectiveness of Mulberry and Red Beetroot as Plaque Coloring on Streptococcus Mutans Glycoprotein

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Abstract

Dental plaque is a thin layer containing various bacteria. Seventy percent of plaque bacteria are the type of Streptococcus mutants. The plaque consists of bacterial glycoproteins that can absorb dyes so that plaque is easily visible. Mulberry and red beet are plants that contain dyes. Mulberry contains anthocyanins, and Red Beetroot contains. In addition to providing red dye, Anthocyanins and betacyanins are also active substances that are soluble in water and can bind to glycoproteins from bacteria. The study aimed to analyze the effectiveness of dental plaque dye based on a combination of mulberry juice and red beetroot on the color absorption in Streptococcus mutants. The type of research is quasi-experimental. This study has two stages, namely in vitro and trials on respondents. Then the data was tested by using the Wilcoxon Sign Rank Test. The results showed that the combination of mulberry juice and red beetroot could stain the glycoprotein of Streptococcus Mutants bacteria at a concentration ratio of 1: 1. The results of hypothesis testing with a p-value (0.813) is 0.05. It means the amount of plaque on the stained tooth surface is no different. In summary, the combination of mulberry juice (Morus alba L.) and red beetroot (Beta vulgaris L.) can replace the function of the disclosing solution.

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Introduction

The level of oral hygiene is generally determined based on the amount of plaque on the surface of the teeth. Plaque tightly attached to the tooth and gingival surfaces has considerable potential for the occurrence of dental caries and its supporting tissues. Plaque is a thin layer containing various bacteria and their products.[1,2] The dominant pathogenic bacteria in dental plaque is Streptococcus mutants. Streptococcus mutants cannot be seen with the naked eye. Streptococcus mutants can be detected in plaque using staining techniques.[1]

In dentistry, a technique for staining Streptococcus mutant bacteria cells in plaque has been developed using a disclosing solution.[2,3] Disclosing solutions in liquid, tablet, or powder form made from chemicals such as Potassium Jodide, Crystallized Iodine, Water, Glycerin, and skinner iodine which are less preferred because of their unpleasant taste, then aniline dyes have been proven potentially cariogenic. The content of erythrosine dye contained in disclosing is a triodine derivative from fluorescein with a high concentration of iodine when ingested, which can cause thyroid cancer.[3,4,5,6]

The use of traditional materials can be used as an alternative to disclosing solutions on the market. Since ancient times there have been many fruits and vegetables that have the potential as a source of natural dyes. Natural dyes can come from fruits and vegetables. One of the fruits that can produce color is Mulberry fruit and Red Beetroot.[7,8,9,10,11,12,13]

Several studies on the features of Mulberry fruit (*Morus alba L*) have been carried out, stating that mulberry contains many chemical compounds, one of which is a benzopyran derivative pigment, namely anthocyanins and flavonoids.[12,14,15,16]

Anthocyanins are often used as red dyes in the field of cosmetics11,12. Anthocyanins are also active substances soluble in water and can

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bind to glycoproteins from bacteria. [17,18,19] Research by Qin et al., 2010 showed that the abundant anthocyanins in mulberry fruit pigments were cyanidin 3-O-rutinoside (60%) and cyanidin 3-O-glucoside (38%).20 Research results of Inhwan et al. 2020 known that in mulberry Morus alba, there are 16 types of anthocyanin substances and the highest level is cyanidin-3-O-glucoside (8.65 mg/g dry weight).21 The results of Zou et al. 2012 study showed that cyanidin-3-glucoside and cyanidin-3-rutinoside is the main anthocyanin in mulberry.[22]

Bindhu and Jayarai, 2020 suggested that the color of anthocyanins is susceptible to pH, light, temperature, and metal ions.[23] Anthocyanin stability is controlled by various factors, including intermolecular and intramolecular complexations. Houghton 2021 stated that as a weak acid, anthocyanins become deprotonated with increasing pH, which can increase electron polarization so that the resulting colors vary, such as red, purple, and blue.

Another type of plant known to contain pigment is the red beetroot. Red beetroot contains a red-violet pigment known as betacyanin.[25,26] The content of betacyanin (purple pigment) and betaxanthin (yellow pigment) contained in beets. These pigments make the color dark red purplish. Because of this intense color, beets are also often used as a natural food coloring.[27,28,29,30]

The results of the research by Setiawan et al. (2015) stated that the analysis of betacyanin content from the skin of red beetroot tubers (Beta vulgaris, L) contained a value of 2.4535mg/100g.31 Other studies that measured betacyanin levels in red beetroot tubers (Beta vulgaris, L) with the addition of various concentrations of ethanol in beetroot and ethanol (50%) is the best concentrate that the betacyanin dye can be obtained optimally in the maceration process.[32] The content of betacyanin (purple pigment) and betaxanthin (yellow pigment) contained in this beet makes it a dark red-purple color. Because of this intense color, beets are also often used as a natural food coloring. Lestari et al., 2016 in their research showed that the betalain content of beetroot was 127.70± 9.34 mg/100g wet weight.33 Boris et al. 2011, stated that the total betanin/isobetanin content in red beet tubers measured spectrophotometrically was (41%), and an increase in neobetanin levels from the chromatographic purification results.[34]

Based on previous research in 2018 regarding "Effectiveness of Mulberry Juice (Morus alba L) on Plaque as a Substitute for Disclosing Solution," the result was that Mulberry juice (Morus alba L) could stain plaque at a concentration of 50% and 100% a layer of purple plaque was seen. The results of staining with Mulberry juice (Morus alba L) were less contrasting when compared to disclosing solution. By combining Mulberry juice with red beetroot juice (Beta vulgaris L), it is hoped that the staining of plaque bacteria will be more contrasting.[35]

Materials and methods

This study uses a quasi-experimental method with a pre and post-design with control group participants—the in vitro research conducted in the laboratory of Poltekkes Bandung. This research has obtained ethical clearance from the KEPK Poltekkes Kementrian Kesehatan Bandung team with Number 03/KEPK/PE/IX/2019. The correspondence test was carried out at the Harapan Elementary School Bandung. The first step of this research is a laboratory test for plaque bacterial staining, and it continues to trial on research samples. After that, the research hypothesis was used a non-parametric method. It is the Wilcoxon Sign Rank Test.

Results

Streptococcus mutants staining using various combinations of Mulberry juice and red beetroot was seen microscopically as a form of the streptococcus group of bacteria (figure 1). The comparison of the combination of Mulberry juice and red beetroot at a concentration of 1:1 showed that the bacteria were spherical, joined together like chains. All groups of bacteria can absorb basic colors, but differences in cell wall thickness cause different colors.

Disclosing solution available in the market was used as a positive control for Streptococcus mutans staining (figure 2). In this process, the bacterial smear that has been fixed is given a dye. Streptococcus bacteria are gram-positive bacteria that have a cell wall composition mostly composed of several layers of petidoglycan. There are about 40 layers of peptidoglycan to form a thick and rigid tang structure.

Streptococcus mutans bacteria in the painting process can retain crystal violet dye, so that microscopically it will look purplish red.

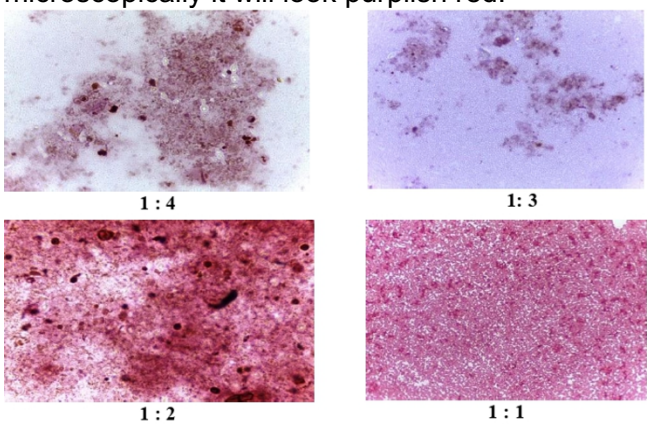

Figure 1. Comparison of bacterial staining with Mulberry (*Morus alba L.*) and Red Beet (*Beta vulgaris L*) juice in various concentrations.

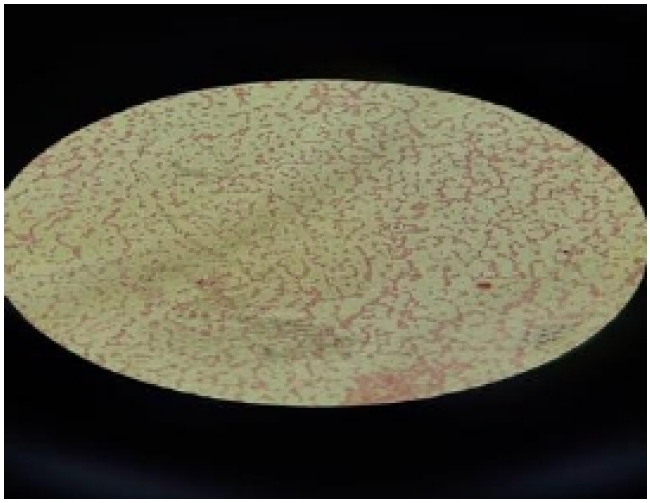

Figure 2. Streptococcus mutans positive control with disclosing solution staining.

The color strength test of the mulberry juice and red beetroot combination was carried out using a spectrophotometer. A spectrophotometer is used because it can measure the absorbance of a dye.[36] This method is quite simple to determine a small quantity of a substance and the results obtained are accurate enough to be directly read by the detector and the results can be obtained in the form of digital numbers.

The results of the color strength test on bacterial staining (table 1) The results of the observation of the color strength test on bacterial staining (table 1) showed the best color absorption of Mulberry juice (Morus alba L.) and Red Beetroot (Beta vulgaris L) by bacterial plaque glycoproteins at a concentration ratio of 1: 1. The result is 0.606, color absorption can only be analyzed after diluting 100 X.

	Sample ID	Type	Ex	Conc	WL478.0	Comments
1	100 + 100 (100	Unk-Repeat			0.606	
2	100 + 100 (100	Unk-Repeat			0.606	
3	100 + 100 (100	Unk-Repeat			0.606	
4	100 + 100 (100	Average		*****	0.606	Avg of preceding 3 Samples
5	100 + 75	Unk-Repeat			0.600	
6	100 + 75-2	Unk-Repeat			0.600	
7	100 + 75-3	Unk-Repeat			0.599	
8	100 + 75-Avg	Average		*****	0.600	Avg of preceding 3 Samples
9	100 + 50	Unk-Repeat			0.458	
10	100 + 50-2	Unk-Repeat			0.458	
11	100 + 50-3	Unk-Repeat			0.458	
12	100 + 50-Avg	Average		*****	0.458	Avg of preceding 3 Samples
13	100 + 25	Unk-Repeat			0.225	
14	100 + 25-2	Unk-Repeat			0.226	
15	100 + 25-3	Unk-Repeat			0.227	
16	100 + 25-Avg	Average		*****	0.226	Avg of preceding 3 Samples

Table 1. The results of measuring the color strength of various mulberry juice (morus alba l.) and red beetroot (beta vulgaris l) comparison on Streptococcus mutans bacteria using spectrophotometry.

The Power of Staining on Dental Plaque

The data on the intensity of the staining strength on the tooth surface using a combination of mulberry juice and red beetroot and the disclosing solution control data are as follows:

Color Strength	Combination of Mulberry Juice and Red Beetroot		Disclosing Solution	
	Frequency	Percent	Frequency	Percent
Strong	47	94.0	50	100.0
Medium	3	6.0	0	0
Total	50	100.0	50	100.0

Table 2. Color intensity distribution.

Table 2 shows that from 50 tooth surface color data using a combination of mulberry juice and red beetroot, 47 (94.0%) staining intensity was included in the strong category.

Figure 3. Comparison of plaque staining combination of Mulberry juice (Morus alba L.) and Red beetroot (Beta vulgaris L.) and Disclosing solution.

Figure 3 shows the strength of the color on the tooth surface stained by Mulberry juice and Red Beetroot juice and using disclosing solution, the red color is almost the same. The combination of Mulberry juice and red beetroot effectiveness test use Wilcoxon Signed Ranks Test. The aim of this test is to compare the data on the number of stained teeth between those using the combination of Mulberry and Red Beetroot and disclosing solution (control data).

	N	Mean Rank	Sum Ranks
m < ds (Negative Ranks)	13	15,88	206,50
m > ds (Positive Ranks)	17	15,21	258,50
m = ds (Ties)	20		
Total	50		

Table 3. Value Ranks Data.

The results of the Wilcoxon signed ranks test showed that from 50 trial respondents there were 13 children have stained teeth surfaces after using a combination of mulberry juice and red beetroot. It was smaller than the number of stained teeth surfaces using disclosing solution. Meanwhile, there were 20 children whose teeth surface stained using the combination of juice (Mulberry and Red Beetroot) and disclosing solution as a positive control was the same. It can be seen that the differences are relatively small. Then the Wilcoxon signed rank test used to see the differences of stained tooth surfaces between those using the juice combination (Mulberry and Red Beetroot) and disclosing solution (control data).

	N	Mean	Deviation Standard	Z	p
Disclosing solution	50	23,28	14,21	-0,543	0,587
Mulberry + Red Beetroot	50	23,34	14,87		

Table 4. Wilcoxon Signed Ranks Test Different Test Results.

Analysis of the Wilcoxon Signed Ranks Test in table 4 shows a p value (0.587) > 0.05. It can be concluded that the number of stained tooth surfaces between those using a juice combination (Mulberry and Red Beetroot) and the disclosing solution control data was not significantly different.

Discussion

The characteristics of bacteria from the genus Streptococcus play a role in dental plaque forming so that Streptococcus mutant bacteria can form extracellular polysaccharides. Plaque bacteria are difficult to see with a light microscope because they cannot absorb or refract light. Therefore, dyes are used to color the bacteria or their background. Living bacteria are almost colorless and contrast with water, where bacterial cells are suspended. One way to observe bacterial cells' shape so they are easy to identify is through painting or staining methods. It also determines its physiological properties for knowing the reaction of the bacterial cell wall through a series of stains. Most bacteria react easily with simple dyes because their cytoplasm is basophilic (like bases), while the dyes used for simple staining are generally alkaline (the chromophoric component is positively charged).[2,3,4,5]

Disclosing solution is a substance that can be used to see whether there is a plaque on the teeth. Moreover, disclosing solution material that is currently commonly used is erythrosine. Erythrosine is a red colorant for food and can also be used to dye bacteria. The glycoprotein contained in plaque can be absorbed by this dye so that the plaque can be seen. This color was chosen because red is easier to see on the teeth

than other colors. However, erythrosine is a triiodide derivative from fluorescein. Its high iodine content can cause thyroid cancer if ingested in large quantities.[4,5,6]

Because synthetic food coloring causes liver damage, it is necessary to find alternative natural dyes. 17 In the modern era, using dyes in food is very popular. The limited quality and sources of natural dyes cause the use of synthetic dyes to proliferate. All types and parts of plants, as well as some microorganisms, can produce dyes. However, the kind of color and chemical compounds contained in it vary depending on the origin of the material and the method of obtaining it. The utilization of pigments produced by plants is still very low at around 7.5%. The remaining 1850 pigments have not been utilized.

Anthocyanin is one of the pigments found in several fruits and vegetable tubers, including mulberry fruit (Morus alba L.) and red beetroot (Beta vulgaris L.).[15,17,37,38,39] Anthocyanins are the most important and most widespread dyes in plants.19 More than 540 anthocyanin pigments have been identified, most of which are structural variations of substituted glycosidic at positions 3 and 5 and possibly acylation of sugar residues with organic acids. 40 This causes anthocyanins to be a source of dye. Anthocyanins are also active substances that are soluble in water and can bind to glycoproteins from bacteria.[8,9,18,24,40] The results of plaque staining from Mulberry (Morus alba L.) and Red Beetroot (Beta vulgaris L) juices with a concentration ratio of 1:1 in this study (figure 1) showed a clearer red-purple color, and this was due to the betacyanin content of the tubers. Red Beetroot (Beta vulgaris L) has a redder color. The concentration of the combination of Mulberry juice (Morus alba L) and Red Beetroot (Beta vulgaris L.), which can absorb the color in the glycoprotein of Streptococcus mutant bacteria, was the most contrasting in the study, namely 1: 1. (Table 1).

The results of this study also showed the strength of the color on the tooth surface stained with Mulberry juice (Morus alba L.) and Red Beetroot (Beta vulgaris L.) and which used disclosing solution. The red color was almost the same (figure 3).

Anthocyanin pigments (cyanidin 3-ramnosyl glucoside 5-glucoside) in mulberry can give a red to purple color.16 Morus Alba L. showed the highest cyanidin-3-O-glucoside content (8.65 mg/g dry weight) among 12 kinds of mulberry fruit. The factor that can inhibit the absorption of paint on bacterial preparations is the high water content (90.2% of the fruit weight) in Mulberry (Morus alba L.), so during the process of rinsing the paint with running water, the paint fades completely.[20,41,42,43,44,45]

Betalain pigments contained in red beets are composed of two pigment compounds, namely reddish-purple betacyanin, and yellowish betaxanthin, which are water-soluble and rich in nitrogen and produce a reddish color so that they have the potential to be used as natural dyes in food products. 46 Betalain pigments can be used as an alternative colorant. The anthocyanins in other types of fruit are due to the stability and resistance of betalains to the influence of pH and temperature, especially at low acidic pH.[47] Betalains are divided into two groups, namely betacyanins with purplish-red pigments and betaxanthins with yellow pigment colors.[48] Betacyanins are dyes. which serves to give red color and can potentially be a natural dye for food that is safer for health than synthetic dyes.[49,50]

Asra et al., 2020, through betacyanin testing carried out by thin-layer chromatography where the Rf value = 0.7166, and a wavelength of 535 nm was analyzed by UV-Vis spectrophotometry method. The FT-IR spectrum showed that the isolate contained functional groups identical to the standard betacyanin (Signs Alderich). Betacyanin was obtained in red beetroot extract with a concentration of 98.65%.[51]

The results of the Wilcoxon Signed Ranks Test in table 5 show a p-value (0.587) > 0.05, so it can be concluded that the number of stained tooth surfaces between those using the combination of Mulberry Juice (Morus alba L) and Red Beetroot (Beta vulgaris L.) and the closed solution control data were not significantly different. Based on these results, it is possible that the combination of Mulberry Juice (Morus alba L.) and Red Beet Tuber (Beta vulgaris L.) at a concentration ratio of 1:1 can be used as an alternative natural dye for Streptococcus mutants Glycoprotein staining so that it can be used as an ingredient. A substitute for disclosing solutions.

According to the research by Qin et al., 2010, the abundant anthocyanin pigments in mulberry fruit pigments are cyanidin 3-O-rutinoside (60%) and cyanidin 3-O-glucoside (38%). Minor anthocyanins (2% total) are pelargonidine 3-O-glucoside and pelargonidine 3-

O-rutinoside. The pigment gives a red to a purplish color.

Nurul et al., 2016 stated that beetroot extract (Beta vulgaris L.) with a concentration of 100% gave a red color more than erythrosine dye for staining plaque in vitro.[52] Research results Setiawan et al. (2015) stated analysis of betacyanin content from beetroot skin Red beetroot (Beta vulgaris, L) has a value of 2.4535mg/100g.[31] Another study that measured betacyanin levels in red beetroot (Beta vulgaris, L) with the addition of various concentrations of ethanol in beetroot and ethanol (50%) was a The best concentrate the betacyanin dye can be obtained optimally in the maceration process.[32] The content of betacyanin (purple pigment) and betaxanthin (yellow pigment) contained in beets is what makes the color dark red purplish. Because of this intense color, beets are also often used as a natural food coloring.

The strength of plaque staining on teeth by Mulberry juice (Morus alba L.) and Red Beetroot (Beta vulgaris L.) was caused by anthocyanin pigments in Mulberry juice (Morus alba L.) and betacyanin pigment from Red Beetroot (Beta vulgaris L.). Betalain pigments and anthocyanins are natural pigments that are soluble in water. Today, the use of these pigments has been developed to replace synthetic dyes that are harmful to human health.[20,50]

Conclusions

The combination of mulberry juice (Morus alba L.) and red beetroot (Beta vulgaris L.) can replace the function of disclosing solution as a dye for Streptococcus mutant bacteria.

Declaration of Interest

The authors report no conflict of interest.

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