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# EFFECT OF FREEZE-DRIED SUPER RED DRAGON FRUIT ANTHOCYANIN DISCLOSING SOLUTION ON DENTAL PLAQUE INDEX

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# ABSTRACT

Background: Accumulation of dental plaque can cause of dental and oral health problems such as caries and periodontitis. Dental plaque is colorless so it is difficult to see visually. Super red dragon fruit can be an alternative disclosing solution because it contains. **Objectives**: The purpose of this research was to determine the content and the effect of anthocyanin disclosing solution freeze dry super red dragon fruit on dental plaque index. Methods: This research is a type of the Experimental Laboratory and Quasi Experimental design research with post-test control group design method. Thirty samples were used and there were three treatment groups: the application of aquadest, super red dragon fruit disclosing solution, and synthetic disclosing solution. Super red dragon fruit freeze dry was tested for antioxidants and tested total anthocyanin levels using the pH difference method at Unjani Pharmaceutical Laboratory. Research on plaque index examination was carried out in the Integrated Dentistry Laboratory Unjani. Dental plaque index assessment is measured using PHPI (Patient Hygiene Performance Index) method. Data were analyzed using Kruskall Wallis and Mann-Whitney tests (p<0.05). Results: The results showed an anthocyanin content of 9.0764 mg/100 g and a significant difference in the anthocyanin disclosing solution freeze dry treatment of super red dragon fruit on the dental plaque index with a value of p = 0.000. Conclusion: In conclusion this research indicates that the freeze-dried super red dragon fruit disclosing solution contains anthocyanin and has an effect on the dental plaque index. This shows the potential of anthocyanin disclosing solution freeze dry super red dragon fruit can be used to detect and control dental plaque, but further research is needed to determine the length of time attached to anthocyanin disclosing solution super red dragon fruit to the tooth surface

Keywords: Anthocyanin, dental plaque, dragon fruit

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# **INTRODUCTION**

The problem of dental health and periodontal diseases begins with the accumulation of dental plaque, which can increase with poor oral hygiene and dietary habits. According to the 2018 Basic Health Research (Riskesdas) data, dental caries cases were reported at 88.8%, and periodontitis at 74.1%. The World Health Organization (WHO) reported in 2018 that half of the world's population, both children and adults, experienced oral and dental diseases. Plaque is a layer that forms on and adheres to the tooth surface, containing oral cavity bacteria. This colonization results in the production of acids, ammonia, carbon dioxide, and oxidants such as hydrogen peroxide. Previous research has identified bacteria in plaque such as S. aureus, S. mutans, Pseudomonas aeruginosa, and E. coli as the main etiology of dental and oral problems.1,2,3

Plaque is colorless or transparent, making it visually imperceptible. Removing plaque from the oral cavity is crucial for preventing dental and oral diseases. Prevention can be achieved by altering dietary habits and controlling plaque mechanically or chemically.<sup>4</sup>

Disclosing solution serves to detect plaque by containing synthetic substances that color the plaque, providing contrast depending on its thickness. The main components of disclosing the solution are erythrosin and basic fuchsin. These synthetic substances, with a cherry-pink color, can stain bacteria, cells, and cytoplasm. Sania's research has highlighted the drawbacks of synthetic disclosing solutions, such as an unpleasant taste due to artificial coloring, potentially causing allergic reactions and acute toxicity.<sup>1,5,6</sup>

The back-to-nature lifestyle is currently trending, with many people cultivating herbal plants for their minimal side effects and effectiveness compared to chemical-containing alternatives. Super red dragon fruit can be an alternative disclosing solution due to its content of betacyanin and anthocyanin, serving as red, purple, and blue colorants.<sup>1,3,7</sup>

Differences in polarity result in electrostatic (protein) and hydrogen bonding (polysaccharide), interacting with anions to produce a color change. The drawback of anthocyanin is its low stability, influenced

by temperature, pH, oxygen, and light. Gusti's research suggests that higher temperatures lead to a decrease in anthocyanin absorbance, indicating instability at high temperatures. Milagros's study supports freeze-drying as an ideal technique for preserving pigments and bioactive substances due to low temperatures.<sup>5</sup>

Based on this, researchers aim to investigate the effect of anthocyanins in super red dragon fruit on dental plaque index and develop a product using natural ingredients as an alternative disclosing solution due to its antibacterial, antioxidant, and non-toxic properties.

#### METHODS

The research was conducted after obtaining ethical approval from the Ethics Committee of Padjadjaran University No. 73/UN6.KEP/EC/2024. This research employs an Experimental *Laboratory* and *Quasi-Experimental* design with a post-test control group design. Purposive sampling technique was used to select samples, which had to meet inclusion criteria and not meet exclusion criteria. with three treatment repetitions. The first treatment involved the use of aquadest, the second treatment utilized disclosing solution from super red dragon fruit, and the third treatment involved the use of synthetic disclosing solution.

#### **Tools and materials**

The materials and equipment used included informed consent, examination sheets, basic diagnostic tools, trays, toothbrushes, writing tools, tissues, analytical balance, measuring glass, cheek retractor, mask, gloves, aquadest, disclosing solution from super red dragon fruit, and synthetic disclosing solution.

#### Sample preparation

Firstly, super red dragon fruits were prepared from the Geulis Garden in Subang, West Java. Subsequently, they were transported to the Pharmacy Laboratory at the Faculty of Pharmacy, Unjani. The fruits were then washed and drained. After draining excess washing water, the super red dragon fruits were finely cut and blend until smooth. A total of 1000 g of super red dragon fruit flesh was mixed with aquadest and left at -40°C for 24 hours in a refrigerated cabinet. It was then transferred to a freeze-drying apparatus at -50°C for 24 hours until a powder was formed. Subsequently, an aquadest with a 2% citric acid concentration and a ratio of 1:4 (material: solvent) was used.<sup>8,9,10</sup>

#### **Polyphenol test**

Polyphenol test was conducted to determine the anthocyanin compound according to Harborne. The polyphenol test involved adding 1 g of freeze-dried super red dragon fruit anthocyanin extract to 5 mL of aquadest and 1 mL of 2M HCl. The mixture was then heated at 100°C for 5 minutes and observed for color changes for 1 minute.

#### Flavonoid test

The flavonoid test was performed by adding 1 g of freeze-dried super red dragon fruit to 5 mL of aquadest, heating the mixture at 100°C for 5 minutes, and then filtering it. The filtrate was combined with 0.1 g of

magnesium powder and 1 mL of concentrated HCl, followed by thorough shaking.<sup>11</sup>

# Determination of total anthocyanin levels using the pH difference method

The determination of total anthocyanin content was conducted using the differential pH method. Each sample, totaling 1 mL, was dissolved in buffer solutions with pH values of 1 and 4.5, each up to 10 mL, resulting in a dilution factor of 10x. Samples dissolved in pH 1 buffer solution were allowed to stand for 15 minutes before measurement, while samples dissolved in pH 4.5 buffer solution were ready for measurement after being mixed for 5 minutes. 1 mL of each liquid extract obtained from maceration was dissolved in the solvent used during maceration to a volume of 5 mL. Subsequently, absorbance was measured in the wavelength range of 400-700 nm using a UV-Vis spectrophotometer. The absorbance of each solution at wavelengths 510 nm and 700 nm was measured, with pH 1 and pH 4.5 buffer solutions used as blanks. The wavelength 510 nm corresponds to the maximum wavelength for cyanidin-3-glucoside, while the wavelength 700 nm is used to correct for any precipitates still present in the sample. The absorbance of the dissolved samples (A) was determined using equation 1, and the total anthocyanin content in the sample was calculated using equation 2.6,10,11

$$A = (Amax - A700)_{pH 1.0} - (Amax - A700)_{pH 4.5}$$
  
Anthocyanin Total (mg/100g) = 
$$\frac{A \times BM \times DF \times 1000}{s \times b}$$

Note:11

BM	=	dilution factor (445.2g/mol)					
DF	=	dilution factor of the sample					
3	=	molar absorptivity of cyanidin-3-					
		glucoside (28.800 L/(mol.cm)					
b	=	cell path length (1 cm)					

All research subjects were instructed not to clean their teeth for 24 hours and were required to record every food and beverage consumed, excluding plain water. Subjects were asked to consume a biscuit 30 minutes before the commencement of the examination.

All research subjects received the same treatment, and each treatment was applied on different consecutive days. On the first day, the treatment involved applying aquadest to the entire surface of the teeth; on the second day, the treatment involved examining the plaque index using freeze-dried super red dragon fruit anthocyanin disclosing solution; on the third day, the treatment involved examining the plaque index using synthetic disclosing solution using Patient Hygiene Performance Index (PHPI) assessment on the tooth surfaces with indices 16, 11, 26, 36, 31, 46 by using a probe and mouth mirror. Data from the treatment of all research subjects were collected for analysis to obtain results for the dental plaque index.<sup>12</sup>

# RESULTS

## Polyphenol and flavonoid test

Antioxidant activity in super red dragon fruit can be elucidated by conducting polyphenol and flavonoid test. According to Harborne (1987), a positive result for polyphenol testing is indicated by the appearance of a green-blue color that fades gradually. Meanwhile, a positive result for flavonoid testing is indicated by the emergence of red, yellow, or orange colors, The results of the polyphenol and flavonoid test analysis on super red dragon fruit confirm the presence of anthocyanins, a class of flavonoid compounds, as shown in Table 1.

 
 Table 1. The result of polyphenol and flavonoid test anthocyanin content of super red dragon fruit

Test	Harborne (1987)	Result
Heated with 2M HCl at 100°C	The green-blue color will slowly fade	(++)
Heated with 0.1 g Mg + 1 ml HCl	Yellow, orange and red color.	(+)

# Determination of total anthocyanin levels using the pH differential methods

In determining the anthocyanin content, the absorbance of each solution at wavelengths 510 nm and 700 nm was measured using pH 1 and pH 4.5 buffer solutions as blanks. The analysis results were obtained using a UV-Vis spectrophotometer in the visible light spectrum. The wavelength 510 nm represents the maximum wavelength for cyanidin-3-glucoside, while the wavelength 700 nm is used to correct for any precipitates still present in the sample. The analysis revealed that the maximum wavelength for anthocyanin is 535,6 nm with an absorbance of 0.911 as shown in Figure 1.



Figure 1. Maximum wavelength of freeze-dried super red dragon fruit 535,6 nm

Based on Figure 1 it is observed that the maximum wavelength is utilized to identify the sample. Anthocyanin compounds exhibit two absorption regions at specific wavelengths, namely UV (278-280 nm) and Visible (465-550 nm). Measurement using a UV-Vis spectrophotometer reveals a peak at the wavelength of

534 nm, indicating the presence of anthocyanin compounds. The predetermined dilution factor is 1, with an obtained absorbance of 0.0543. The total anthocyanin content of freeze-dried super red dragon fruit can be observed in Table 2.

**Table 2.** The total anthocyanin levels of super red dragon fruit

	Total			
pH 1		рН 4.5		Anthocyanin
λ534	λ70	λ534.	λ70	Content
.6 nm	0 nm	6 nm	0 nm	(mg/100g)
0.14	0.0	0.10	0.0	0.0674
4	27	7	44	9.0074

Based on the calculation results, the determined total anthocyanin content is 9.0674 mg/100g. The total anthocyanin levels of freeze-dried super red dragon fruit is higher than the total anthocyanin levels of white dragon fruit according to the study conducted by Mimi et al.

Data distribution in each treatment group was analyzed for the normality using *Saphiro-Wilk* test and homogeneity using *Levene's* test. Normality tests on the treatment groups of aquadest and freeze-dried super red dragon fruit showed a p value p<0.05, therefore data were distributed non-normally. Meanwhile, the treatment group with synthetic disclosing solutions were distributed normally.

The homogeneity test results indicate the data showed a p value >0.05 is not homogeneous. Therefore, for the analysis of the anthocyanin content of freezedried super red dragon fruit disclosing solution on dental plaque index, a non-parametric statistical analysis using Kruskal-Wallis, the administration of aquadest, super red dragon fruit disclosing solution, and synthetic disclosing solution shows significant results on the dental plaque index of the subjects. The research findings involving 30 students from the Faculty of Dentistry at Universitas Jenderal Achmad Yani indicate that freeze-dried super red dragon fruit anthocyanin disclosing solution has an effect on dental plaque index by detecting and reducing plaque on the tooth surfaces. The obtained significance with a p-value 0.000 is presented in Table 3.

**Table 3.** The effect of freeze dried super red anthocyanin disclosing solution on dental plaque index

Group Test	Dental Plaque Index	<b>P-Value</b>
Aquadest	1.600±0.829	
Freeze-dried super red dragon fruit	2.557±0.546	0.000
Synthetic disclosing solution	1.887±0,320	_

According to Table 3, the obtained p-value for the dental plaque index is 0.000 (<0.05). This indicates a

significant difference among the treatment groups using the freeze-dried super red dragon fruit anthocyanin disclosing solution in relation to the dental plaque index. The aquadest group has a mean of 1.600, categorized as good; the super red dragon fruit freeze-dried disclosing solution group has a mean of 2.557, categorized as moderate; and the synthetic disclosing solution group has a mean of 1.887, also categorized as moderate. Based on these results, the freeze-dried super red dragon fruit disclosing solution is more effective in detecting plaque compared to aquadest, and it can reduce the dental plaque index after examination using synthetic disclosing solution.

### DISCUSSION

Polyphenol and flavonoid test is useful for identifying the compounds present in the super red dragon fruit, and this is influenced by the solvent used. In the polyphenol and flavonoid test results for super red dragon fruit with 2M HCl, heated to 100°C, the intense bluish-green color produced indicates the presence of anthocyanin compounds. After heating, 0.1 g of Mg powder and 1 mL of concentrated HCl are added, then the mixture is shaken, resulting in a faded red color. Thus, the test results align with Harborne's theory.<sup>5,10,11</sup>

The accumulation of plaque contains bacteria, and if plaque buildup continues, it can lead to issues in the oral cavity, such as tooth decay, tartar formation, bad breath, gum inflammation, and oral cavity infections. The use of synthetic disclosing solution can be employed to detect plaque. The staining mechanism of plaque in synthetic disclosing solution occurs due to differences in polarity. The glycoprotein present in dental plaque is an oligosaccharide protein that binds glycan with covalent bonds. Differences in polarity result in electrostatic (protein) and hydrogen bonding (polysaccharide), allowing functional groups to interact with anions, leading to a color change. The drawback of synthetic disclosing solution is its content of toxic chemicals and unpleasant taste. Natural pigments like anthocyanins are safe for the body as they originate from plants, such as the super red dragon fruit.<sup>13,14,15</sup>

According to Hong's research, anthocyanin compounds can promote health due to their antioxidant properties, potentially acting as anticancer, antidiabetic, antiobesity, antimicrobial, cardioprotective, neuroprotective agents, and reducing oxidative stress. Anthocyanins are compounds that produce blue, red, or purple pigments derived from plants, such as flowers, tubers, and fruits. One of the plants used in this research is the super red dragon fruit (Hylocereus costaricensis). Under acidic conditions, anthocyanins appear as redpigments because of the flavylium cation produced at low pH, while under basic conditions, they appear as blue pigments. The stability of anthocyanins is influenced by pH, light, temperature, and structure.<sup>16</sup>

Anthocyanins have two components: sugar and polysaccharide. The polysaccharide in anthocyanins binds with the polysaccharide in plaque, allowing the detection of plaque through changes on the tooth surface.  $^{17,18,19} \,$ 

Polyphenol and flavonoid tests are useful to determine the content present in compounds within the super red dragon fruit, influenced by the solvent used. In the polyphenol and flavonoid test results on super red dragon fruit with 2M HCl, then heated at 100°C, the resulting color is a dark bluish green, indicating the presence of anthocyanin compounds. In the second method, after heating, 0.1 g of Mg powder and 1 mL of concentrated HCl are added, then shaken. The resulting color is a faded red, aligning with Harborne's theory.<sup>10</sup>

Measurement using a UV-Vis spectrophotometer is useful to determine the maximum wavelength of anthocyanins in freeze-dried super red dragon fruit, thus determining the total anthocyanin content. Anthocyanins have a range of 400-700 nm. The maximum wavelength determination with a UV-Vis spectrophotometer reveals a peak at 510 nm, indicating the presence of anthocyanin compounds. In Amaliya's research, the absorption spectrum of anthocyanin compounds in 100% super red dragon fruit extract has a maximum absorption at a wavelength of 500 nm for the first line indent.<sup>13,20</sup>

The next step is to measure freeze-dried super red dragon fruit using a UV-Vis spectrophotometer with the differential pH method to observe the comparison of anthocyanin content tested at different pH levels, namely pH 1 and pH 4.5. Based on the study, anthocyanin color is influenced by pH due to the ionic nature of the molecular structure. At pH 1-4, the color of anthocyanin compounds is stable, while at pH 4-5, it is unstable. The total anthocyanin content in freeze-dried super red dragon fruit based on the differential pH formula is 9.0674 mg/100g. If the absorbance value is high, the total anthocyanin content will also be higher, as mentioned in the introduction.<sup>21</sup>

The research results on 30 students of the Faculty of Dentistry, General Achmad Yani University, show that freeze-dried super red dragon fruit anthocyanin disclosing solution affects the dental plaque index by detecting and reducing plaque on the tooth surface. Based on the non-parametric Kruskall-Wallis statistical analysis, the administration of water, super red dragon fruit disclosing solution, and synthetic disclosing solution significantly affects the dental plaque index of subjects.Significant results with a pvalue of <0.05 can be seen in the Discussion and should refer to at least Table 3.

A good plaque disclosing agent should have a pleasant or acceptable taste for patients, exhibit high color intensity in the oral cavity, and not easily fade. In this study, the disclosing solution from super red dragon fruit adhered for only 15 seconds. The brief attachment duration is due to the anthocyanin pigment content in super red dragon fruit, which easily dissolves in water, resulting in shorter adhesion.<sup>22,23</sup>

Disclosing solutions made from the peel of super red dragon fruit can be formulated as a gel. Gels possess stability and an attractive appearance. The stability of the gel ensures the integrity of the active compound content. Research conducted by Luh indicates that the gel formulation has good spreading ability to release its active ingredients. Based on Eky's study in 2020, a gel formulation with 15% super red dragon fruit extract can be used as a natural dental plaque disclosing solution. The capability of the gel extract from super red dragon fruit effectively stains dental plaque, covering 40.32% of the total plaque on the tooth surface. According to Sophia's research, the dragon fruit extract used as a natural disclosing solution obtained results in organoleptic testing, stating that the dragon fruit preparation has a sweet and fruity taste.<sup>24,25,26</sup>

In this study, freeze-dried super red dragon fruit disclosing solution was supplemented with 2% citric acid, which functions to stabilize the color. The concentration of citric acid affects the total anthocyanin content in super red dragon fruit. Citric acid regulates pH for pigment release and preservation. Citric acid can protect pigments from damage caused by oxidation. An increase in pH can cause anthocyanin pigments to fade, so the addition of 2% citric acid was performed as it represents the optimum point for anthocyanin. The addition of citric acid to freeze-dried super red dragon fruit can impact the stability of anthocyanins but may not provide longer staining duration compared to the disclosing solution.<sup>8,20,27</sup>

Based on Eka's bacterial inhibition testing research, the extract dissolved in 5% acetic acid can inhibit the growth of Staphylococcus aureus. The addition of acetic acid can be considered as it may act as an antibacterial agent by denaturing proteins, leading to the cessation of bacterial cell metabolism activities.<sup>27,28,29</sup>

From Table 3, significant differences are observed in the super red dragon fruit disclosing solution, indicating its effectiveness in reducing the dental plaque index. According to Arina's research, apart from flavonoid compounds, there are other antibacterial compounds, namely alkaloids found in the peel of this fruit, known as betacyanins.

Alkaloids have a mechanism to inhibit bacterial growth by disrupting the components of peptidoglycan in the bacterial wall, making it unstable, and inhibiting the activity of the enzyme dihydrofolate reductase in bacteria, thereby impeding nucleic acid synthesis.<sup>22,26</sup>

Several factors can influence the outcomes of the disclosing solution. Saliva plays a crucial role in the oral cavity, functioning to minimize dental plaque growth. Poor oral hygiene conditions with low saliva flow contribute to a higher plaque amount in the mouth. Based on the research conducted by Amaliya, dragon fruit can be used for plaque identification on tooth surfaces due to the presence of betacyanin compounds, which exhibit a reddish-purple color, making it an alternative natural color.<sup>30,31</sup>

Additionally, research by Diyah indicates that betacyanin is found in beets, a water-soluble

compound with antimicrobial and antiviral effects. Susana's study, using apples to reduce dental plaque and saliva bacteria indices, found that chewing apples did not change the dental plaque index but resulted in a decrease in saliva bacteria.<sup>5,22,32</sup>

Limitations in the research include time and cost constraints, leading to the inability to control all variables that may affect the study results effectively. Variables such as eating habits, oral hygiene routines, or dental history were not fully controlled, providing a more comprehensive picture. The compound anthocyanin is found in super red dragon fruit with a total anthocyanin level of 9.0764 mg/100 g and the anthocyanin compound content in the freeze-dried disclosing solution of super red dragon fruit has an effect on detecting and reducing the dental plaque index.

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