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**THE EFFECT OF ROSELLE (*Hibiscus sabdariffa* L.) PETALS EXTRACT AS  
ALTERNATIVE DISCLOSING SOLUTION FOR DENTAL PLAQUE IDENTIFICATION**

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**ABSTRACT**

**Background:** *Roselle (Hibiscus sabdariffa L.) is a plant with many functions, such as natural dyes. In dental health, disclosing agents are being used to identify the amounts of dental plaque by showing contrast colour of dental plaque. One of disclosing agent which often being used is disclosing solution. Purpose:* To know the effect of roselle petals as alternative substitute disclosing solution to identify dental plaque with contrast color of plaque. **Method:** Research design was *Quasi Experimental with Post Test Only Control Group Design. Subject was 45 people which divided into 3 groups. Group 1 was 15 people given roselle petals extract concentration 50%, Group 2 was 15 people given roselle petals extract concentration 100% and group 3 (control) was 15 people given disclosing solution. Result:* The study results showed that the mean of plaque index after application of roselle petals extract concentration 50%, 100% and disclosing solution was 1,720, 1,951, 2,140. The data was tested using *One Way ANOVA* which was resulted in 0,018 significancy ( $p < 0,05$ ). There was significant differences of plaque index after three groups of application. **Conclusion:** There is significant effect of roselle petals extract concentration 100% as an alternative disclosing solution to determine dental plaque.

**Keywords :** *Dental Plaque, Disclosing Solution, Roselle Petals Extract*

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**INTRODUCTION**

Dental and oral health for some people, especially in Indonesia, remains an umpteenth priority. According to the Riset Kesehatan Dasar (Riskesdas), in 2007 and 2013, there was an increase in dental and oral health problems from 23% to 26% where 14 provinces had a prevalence of dental and mouth problems higher than the national average. Moreover, people who brushed their teeth correctly only 2.3%. It shows that people have a low awareness in keeping their dental hygiene. The impact of the people who have poor brushing habits may lead accumulation of

plaque on the areas that are not brushed and lead to the occurrence of dental and oral health problems such as caries and gingivitis.<sup>1,2</sup>

Plaque is a biofilm consisting of 70% of bacteria and 30% of organic and inorganic intracellular matrices attached to the tooth surfaces. The formation of plaque on the tooth occur in three stages. The first stage is the formation of dental pellicle i.e. a thin organic layer (0.5  $\mu\text{m}$ ) consisting of proteins and glycoproteins attached to the tooth surface and formed a few minutes after the tooth surface is completely cleansed and in contact with the saliva. Secondly, initial colonization of

bacterial by attachment to the pellicle adhesively within 3-4 hours. The bacteria that dominate are the gram positive bacteria such as *Streptococcus sanguis* and *Streptococcus mutans*. There is an increase of plaque around two to four days and the plaque maturation occurs on the seventh day with the increase of gram negative bacteria.<sup>3,4,5</sup>

Plaque cannot be seen directly by our eyes. Therefore, it needs dyes substance also known as disclosing agent. Disclosing agent has various dosage forms ranging from solution, tablet, gel. Disclosing agent which often used is in the form of solution. One of disclosing solution compositions is the content of synthetic dyes such as erythrosine and basic fuchsin. Based on Acceptable Daily Intake (ADI), maximum dosage of erythrosine synthetic dye that is not dangerous if swallowed is 0,0-0,6 mg. The side effects of ingesting this synthetic dyes in high dosage can result in carcinogenesis, in addition, it can cause allergic reactions such as shortness of breath, chest pain, headache, and skin irritation.<sup>6</sup> Dental plaque control can be done in two ways. First, mechanical plaque control with brushing teeth. Second, chemical plaque control with mouthwash.<sup>4</sup>

Ristania (2010) stated that the red fruit have better quality in coloring plaque than the disclosing solution with synthetic dyes.<sup>7</sup> The roselle petals (*Hibiscus sabdariffa* L.) are known by the society as a plant that is used as a traditional medicine and as a natural dyes. The red color of roselle petals is derived from anthocyanins pigments.<sup>8</sup> Roselle petals contain active substances such as flavonoids, tannins, phenols or polyphenols, citric acid, saponins and anthocyanins pigments. Those active are beneficial to the health of the body.<sup>9</sup> Flavonoid can inhibit the growth of microorganisms such as *Streptococcus mutans* by forming complex compounds with proteins through hydrogen bonds. Tannin can inhibit the production of microbial enzymes binding for microbial adhesion and bacterial cell walls. Phenol or polyphenols serves as antibacterial by altering cell proteins and destroying bacterial plasma

membranes. Saponin can inhibit microbial growth by interacting with the sterol membrane. These anthocyanins pigments provide a reddish purple on the roselle petals.<sup>10</sup> The purpose of this study is to know the effect of roselle petals extract as an alternative natural dyes for disclosing solution in identifying dental plaque.

## MATERIALS AND METHODS

This study has used Quasi Experimental with Post Test Only Control Group Design. This study protocol were approved by the Research Ethical Committee of Faculty of Dentistry, Islamic University of Sultan Agung-B033/B.1-KEPK/SA-FKG/IV/2017. The research subjects are 45 the college students. The subjects obtained was used Lemeshow method. The subjects has been accepted the inclusions and exclusions criteria. The subjects has obtained the information sheet and signed the informed consent as the subject of the research. The subjects were divided into three groups using simple random sampling and each group consists of 15 people. Group 1 was roselle petals extract treat extract with concentration 50%. Group 2 was roselle petals extract treatment with concentration 100% and group 3 was disclosing solution with fuchsin synthetic dyes. Meanwhile, independent variable were roselle petals extract and disclosing solution synthetic dyes, dependent variable were dental plaque index (PHP method).

The instrument used in the extraction process of roselle petals extract were bowls, steamer, dan glass funnel. The instrument used in examination for dental plaque were dental instrument, neirbekken, dappen glass, masks, handsoons. The material used in examination for dental plaque were roselle petals extract concentration 50% and 100%, disclosing solution fuschin synthetic dyes, cotton pellets, cotton rolls.

The research began with the extraction of roselle petals. The seeds of roselle were disposed and fresh roselle petals were sliced.

The roselle petals were then blanched with temperature of 60 degree celcius for 5 minutes. The roselle petals were dried in air drying with ratio dry:wet = 10:1, then froze using freeze dryer for 24 hours to be more durable, dry and easily mashed. The roselle petals were dried and mashed using a blender so be roselle petals powder could be obtained. The extraction of roselle petals powder with solvent: Aquadest+Citrate Acid 2% with ratio 10:1, continued with maceration for 24 hours at 4°C and in dark condition (Erlenmeyer coated with aluminum foil). After obtained, extract roselle petals concentration 100%, then diluted using sterile aquadest to obtain roselle petals extract concentration 50%. Then, roselle petals extract concentration 50% and 100% were tested including ph test using digital pH, level of anthocyanins test using spectrophotometer, and color test using colorimetric.

Before the examination of dental plaque, subject were standardized by giving intruction for brushing teeth with stillman, roll, and bass methods for two minutes. Then, subjects were instructed to eat cariogenic foods (chocolate, caramel waffer) prior to the examination. It was aimed to equate the subject oral condition before treatment. The required materials on dental plaque examination were extract roselle petals concentration 50%, extract roselle petals concentration 100%, and disclosing solution synthetic dyes. The subjects were divided into 3 groups randomly to the treatment groups. Group 1 was extract roselle petals concentration 50%, group 2 was extract roselle petals concentration 100%, and group 3 was disclosing solution synthetic dyes.

The examination of dental plaque was conducted four hours after subject get cariogenic food. This was because dental plaque is formed ± 3-4 hours after their the teeth are exposed cariogenic foods. The operators was recording the examination results on the dental plaque index examination sheet using the Patient Hygiene Performance Index (PHP Index) from Podshadley and Haley (1968) cit. Putri MH (2010).

The tooth surface were examined on the predetermined six crowns are 11 labial, 16 buccal, 26 buccal, 31 labial, 36 lingual, 46 lingual by dividing each tooth surface into five subdivisions, D (distal), G (middle third of gingiva), M (mesial), C (middle third), O/I (one third of incisal or occlusal). Then, the result of plaque index was measured. The measurement criteria for plaque measurement was 0 when free of plaque (if it is free of disclosing solution attached to the tooth surface) and 1 when there was a plaque (if there is a disclosing solution attached to the tooth surface). In assessing the results of dental plaque examination to determine the plaque index of PHP, these formulas were used:<sup>5,11</sup>

$$IP\ PHP = \frac{\text{Total number of surfaces containing plaque}}{\text{number of teeth examined}}$$

Plaque index of PHP was divided into several categories based on the severity: Good (0,1-1,7), Fair (1,8-3,4), and Poor (3,5-5,0).<sup>5</sup> The data obtained were analyzed statistically and presented in table form. The data was tested with normality test using Shapiro Wilk resulting in  $p > 0,05$  and homogeneity test using Levene's test resulting in  $p > 0,05$ .

## RESULTS

### Test Result of Roselle Petals Extract

Table 1. Roselle Petals Extract

Type of test	Result		
	1	2	3
Levels of Anthocyanin(mg/L)	83,45	106,29	0
pH	0,98	0,61	8,15
Colour:			
L* (Lightness)	29,68	26,07	30,67
a* (Redness)	3,67	2,40	2,42
b* (Yellowness)	-1,28	-0,03	-0,23

Description:

Group 1 = Roselle Petals Extract concentration 50%

Group 2 = Roselle Petals Extract concentration 100%

Group 3 = Disclosing solution fuchsin syntetic dyes

According to the table above, levels of anthocyanin shows that roselle petals extract concentration 100% (106,29) has the highest level of anthocyanins than concentration 50%. But, disclosing solution syntetic dyes (0,00) has no anthocyanins content.

Result of pH test shows that roselle petals extract concentration 100% has acidic pH (0,61), roselle petals extract concentration 50% has acidic pH (0,98), and disclosing solution has alkaline pH (8,15).

Result of color test shows that roselle petals extract concentration 100% has red color (approaching disclosing solution color) and darker than roselle petals extract concentration 50% which has purplish red color. While disclosing solution shows red color, according to picture 1.

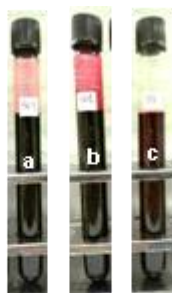


Figure 1. Sample (a). Roselle petals extract concentration 50%, (b) . Roselle petals extract concentration 100%, (c) Disclosing Solution fuchsin syntetic dyes

### Result of Dental Plaque Examination

Table 2. Data Description of dental plaque Examination

Numb.	Criteria	1		2		3	
		N	%	N	%	N	%
1	Good (0,1-1,7)	5	33	2	13	0	0
2	Fair (1,8-3,4)	10	67	13	87	14	93
3	Poor (3,5-5,0)	0	0	0	0	1	7
<b>Total</b>		15	100	15	100	15	100
<b>Mean number of Dental Plaque Index</b>		1,720		1,951		2,140	

Description:

Group 1 = Roselle Petals Extract concentration 50%

Group 2 = Roselle Petals Extract concentration 100%

Group 3 = Disclosing solution fuchsin syntetic dyes

The data obtained from table 2 shows the highest percentage of dental plaque index examination in group 1 was in poor category with total number eight subjects (53%). The highest percentage of dental plaque index examination in group 2 was also in poor category with total number of nine subjects (60%). The highest percentage of dental plaque index examination in group 3 was in poor category with total number of eleven subjects (73%).

Based on the table, it shows that there is significant difference in the mean of dental plaque index between roselle petals extract concentration 50% (1,720), roselle petals extract concentration 100% (1,950) and disclosing solution fuchsin syntetic dyes (2,140). From the data obtained, the statistical analysis can be done between the three groups.

Table 3. Normality dan Homogeneity Test

	Sig.	
Test of Normality (Shapiro Wilk)	Group 1	0,600
	Group 2	0,576
	Group 3	0,110
Homogeneity of Variances (Levene's Test)	0,570	

According to the table 3 above, the normality test using Shapiro Wilk shows the significant difference value of plaque index between roselle petals extract concentration 50% (0,600), roselle petals extract concentration 100% (0,576), and disclosing solution (0,110). The data was normally distributed because the significance value was more than 0,05 ( $p > 0,05$ ). The homogeneity test using Levene's test shows that the data significance value was 0,570 ( $p > 0,05$ ). The result shows the data are homogeneous. So, statistical data test were carried out using One Way ANOVA to find out whether there were significant differences between three groups with 95% confidence level.

Table4. *One Way ANOVA*

	F	Sig.
<i>One Way ANOVA Test</i>	4,397	0,018

Table 6 shows that there was an effect of roselle petals extract concentration 50% and 100% against the average amount of plaque index with significant value 0,018 ( $p < 0,05$ ). It was continued with *Post Hoc LSD* to find out significant difference between groups with significant value of  $p < 0,05$ .

Table 5. *Post hoc LSD*

Group		Sig.
Group 1	Group2	0,121
	Group 3	0,005
Group2	Group 1	0,121
	Group 3	0,175
Group3	Group1	0,005
	Group2	0,175

Table 5 shows that group 1 and 2 has no significant difference with  $p = 0,121$  ( $p > 0,05$ ), group 1 and 3 has significant difference with  $p = 0,005$  ( $p < 0,05$ ), group 2 and 3 has no significant difference with  $p = 0,175$  ( $p > 0,05$ ). It can be concluded that roselle petals extract concentration 100% have the similar effect with disclosing solution as dyes to determine dental plaque.

## DISCUSSION

According to the test result of anthocyanins content, it shows that roselle petals extract concentration 100% has the highest level of anthocyanins than concentration 50% whilst disclosing solution has no anthocyanins content because of the materials used to disclose dental plaque i.e synthetic dyes. Anthocyanins is natural pigments, which are red, purplish red, dark red that commonly found in plant organs such as flowers, leaves, and fruits.<sup>12</sup> It is natural dyes substances which is classified into benzopyran derivatives. The main structure of the benzopyran derivative is characterized by the presence of two aromatic rings of benzene ( $C_6H_6$ ) with three carbon atoms which form the ring. It can be used as natural dye and soluble

in water. It is usually found on flowers, leaves and fruit of plants.<sup>13</sup>

According to Dian in Satyatama (2008) anthocyanins dyes was not changed and showed purplish red color.<sup>14</sup> The content of anthocyanins in roselle petals extract has similar quality of disclosing solution synthetic dyes to disclose dental plaque with binding glycoprotein in dental plaque. Application of roselle petals extract is better than disclosing solution (synthetic dyes) because it attaches to dental plaque and can be cleaned easily from the teeth. Roselle petals extract is safe when ingested whilst disclosing solution with synthetic dyes is suggested not to be ingested. According to Nurnasasi and Ahmad (2017), natural pigment such as anthocyanin as food grade dye is safe when ingested, while synthetic dyes can be carcinogenic.<sup>9</sup>

The result of pH test shows that the roselle petals extract concentration 50% (0,98) and roselle petals extract concentration 100% (0,61) have an acidic pH, whilst disclosing solution synthetic dyes (8,15) has an alkaline pH. This is because the roselle petals have a sour taste that indicates low pH (acid). The roselle petals extract uses aquadest + citric acid 2% for solvent. Citric acid is able to maintain the stability of the anthocyanins content. Citric acid itself has the ability to increase the degree of acidity (pH). If teeth are exposed to acid for too long, it can affect the enamel layer of the tooth and can increase the risk of dental caries. Tooth enamel damage due to demineralization acid is affected by time ( $\pm 24$  hours) and frequency of exposure (mild 1-7 times/week, moderate 8-21 times/week and severe  $\geq 22$  times/week). The occurrence of dental caries process is related to four main factors: host, agent, substrate and time. The development of dental caries takes up to several months. Meanwhile, the application of roselle petals extract is only used once a while and less than five minutes. Saliva in the oral cavity has a role to prevent tooth decay in few days or few weeks. So the acid on the roselle petal extract does not included in the factors that cause dental caries (time).<sup>15,16,17</sup>

The test result of One Way Anova shows significance value of 0,018 ( $p < 0,05$ ), which means that there was a significance difference between the treatment groups. This result means that there may be similarity in the disclose activity between two groups to disclose dental plaque. Post Hoc LSD Test was performed to find out the significant difference between the groups. It is known that roselle petals extract concentration 100% have the similar effect with disclosing solution if compared with roselle petals extract concentration 50% as dyes to disclose dental plaque. This is probably because the level of anthocyanins content (red dyes). Roselle petals extract concentration 100% has the highest level of anthocyanins which make a significant difference in disclosing dental plaque.

pH changes can change the color of anthocyanins. When pH is increase the color of anthocyanins will be violet and will be red while pH decrease. The occurrence of stable red color is because of the dominant number of methoxy groups versus hydroxy groups in the anthocyanin structure. In addition to the influence of pH, anthocyanins dyes affected by pigment concentration and the presence of mixtures with other pigments (copigmentation) as well as the formation of bonds with sugar particles or colloid.<sup>14</sup> It can be concluded that the roselle petals extract concentration 100% has an effect as a natural dyes to identify dental plaque. The roselle petals have some advantages, one of them is the presence of antibacterial content which is not obtained from the disclosing solution of synthetic dyes.

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