



## Effectiveness of Rosella Flower Extract (*Hibiscus Sabdariffa* L.) in Gel and Liquid form on the Growth of *Streptococcus Mutans* Bacteria

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[Original Article](#)

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### ABSTRACT

**Background:** The most dominant bacteria in dental caries-causing plaque is *Streptococcus mutans* bacteria, which is a type of acid-producing bacteria that can damage teeth due to the fermentation reaction of carbohydrates including sucrose, fructose, and glucose. Inhibiting the growth of these bacteria is an alternative way to inhibit the growth of plaque so as to prevent dental and gum disease. One of the herbal plants that can be used as an alternative medicine as an anti-bacterial is rosella flower (*Hibiscus sabdariffa* L.)

**Material and Methods:** The research design used is Experimental. The research was conducted from July to November 2021 at the Pharmacognosy Laboratory, Department of Pharmacy and Food Analysis, Poltekkes Kemenkes Jakarta II, and QLab, Faculty of Pharmacy, University of Pancasila. The research sample was rosella flower extract in gel and liquid preparations in concentrations of 5%, 10%, 15%, 20%, and 30%. Data analysis using Repeated Measure Anova and Independent Sample Test.

**Results:** Rosella flower extract was effective in inhibiting the growth of streptococcal mutans bacteria, where the gel preparation had a p-value of 0.000 ( $p < 0.05$ ) and a liquid preparation with a p-value of 0.003 ( $p < 0.05$ ). The average zone of inhibition of the growth of *Streptococcus mutans* bacteria in gel preparations was greater than in liquid preparations in each concentration. The zone of inhibition for gel preparations at 30% concentration was 14.4 mm, while for liquid preparations it was 10.7 mm. The smallest inhibition zone was gel preparation at a concentration of 5% of 10.8 mm, while the smallest liquid preparation was at a concentration of 10% at 6.8 mm.

**Conclusion:** Rosella flower extract was effective in inhibiting the growth of streptococcus mutans bacteria and rosella flower extract in gel form was greater than liquid in inhibiting the growth of streptococcus mutans bacteria.

**Keywords:** Gel, Liquid, Rosella Flower Extract, *Streptococcus Mutans*.



## Introduction

Dental caries is the most common dental disease in the community. Caries are a disease that begins to attack the enamel and dentin, which is caused by bacterial activity. The main cause of the formation of dental caries is due to the presence of a soft deposit attached to the teeth consisting of various kinds of bacteria, called plaque.<sup>1-3</sup>

The most dominant bacteria found in caries-causing plaque is *Streptococcus mutans*, a type of acid-producing bacteria that can damage teeth due to carbohydrate fermentation reactions including sucrose, fructose, and glucose. Inhibiting the growth of these bacteria is an alternative way to inhibit the growth of plaque so as to prevent dental and gum disease.<sup>4-6</sup>

There are many problems that arise due to the current use of chemical/synthetic drugs, it is necessary to find new alternatives that are more natural in nature and which are not expected to have side effects on host/human cells. One of the herbal plants that can be used as alternative medicine as an anti-bacterial is Rosella (*Hibiscus sabdariffa* L.). In scientific research, rosella flowers contain important chemical substances, including gossypetin anthocyanins, glucoside hibiscin, arginine, legnin, vitamin C, calcium, organic acids, polysaccharides, and flavonoids.<sup>7-9</sup>

Drink ingredients from rosella recently began to be widely known as a health drink. In many countries, the use and efficacy of rosella in the world of medicine are no stranger. In India, Africa, and Mexico, all parts of the roselle plant serve as traditional medicine. Rosella plants have antiseptic, anti-bacterial, anti-hypertensive, diuretic effects, improve blood circulation, antispasmodic, antihelminthic, treatment of bleeding gums, and increase the human body's resistance to disease, etc. Other properties as an anticonvulsant (antispasmodic) treat intestinal worms (antihelminthic), and an antibacterial. Rosella extract has a lethal effect against *Mycobacterium tuberculosis* which causes tuberculosis. Another benefit of the rosella plant is to reduce blood viscosity and as an intestinal antiseptic.<sup>10-13</sup>

One of the efforts to prevent dental and gum disease using natural ingredients, it is necessary to carry out an Anti-bacterial Test of Rosella Flower Extract (*Hibiscus sabdariffa*) against mutant *Streptococcus* bacteria in Gel and Liquid dosage forms as a continuation of the early-stage research on the antibacterial effect of Rosella flower extract. The concentration of the extract used is ranging from 5% to 30%.<sup>14</sup>

## Materials and Methods

This research is an experimental study, to test the comparison of the effectiveness of Rosella Flower Extract (*Hibiscus sabdariffa* L.) in the form of gel and liquid against the inhibition of *Streptococcus mutans* bacteria. The research will be conducted from July to November 2021 at the Pharmacognosy Laboratory Department of Pharmacy and Food Analysis, Poltekkes Kemenkes Jakarta II and QLab Faculty of Pharmacy, University of Pancasila. Independent variables: rosella flower extract in the form of gel and liquid concentrations of 5%, 10%, 15%, 20%, 30%. As a positive control, Amoxicillin was used while the dependent variable was the growth of *Streptococcus mutans* bacteria by measuring the zone of inhibition. This research procedure is carried out in several stages:

### 1. Making ethanolic extract of rosella flower in liquid form

Preparation of ethanol extract of Rosella Flower in liquid form was obtained by maceration method using 95% alcohol solvent in a ratio of 1:5. Simplicia and solvent were immersed in a vessel for 4 days. The filtrate and the dregs were separated with a Buchner equipped with a vacuum. The pulp was macerated 2 times. The

filtrate is concentrated with a rotary evaporator and a water bath. The stages of making rosella flower extract are as follows:

- a. The collection of rosella flowers (*Hibiscus sabdariffa* L.) was carried out in the morning and then the leaves were washed under running water until clean, drained, thinly sliced, and dried by aerating.
- b. The dried samples were crushed using a blender, the resulting powder was sieved to obtain a fine and uniform powder. The results are put in a closed container.
- c. Rosella flower extract was made by maceration method. A total of 60 grams of rosella flower simplicia powder was put into an Erlenmeyer, then soaked with 225 ml of 95% ethanol solution, covered with aluminum foil and left for 5 days while stirring occasionally.
- d. After 5 days, the sample was filtered using filter paper to produce a liquid filtrate.
- e. The filtrate was evaporated using a rotary evaporator to obtain a thick extract of miana leaves. The thick extract was left at room temperature until all of the ethanol solvent had evaporated. The extracts were weighed and stored in a closed container before being used for testing.

## 2. Making a variety of extracts

Calculation of the dilution of the test extract using the formula:

$$V1.M1 = V2.M2$$

### Information:

- V1 : Initial volume of roselle flower extract  
M1 : Initial concentration of rosella flower extract  
V2 : Final volume of roselle flower extract  
M2 : Final concentration of rosella flower extract

The dilution of the test extract was carried out by mixing the test extract with DMSO. The final volume of concentrations of 5%, 10%, 15%, 20%, 30% is liquid to 10 ml.

## 3. Making rosella flower extract gel with carbomer base

Carbomer weighing 2 grams per formulation was dispersed in distilled water (10 times the amount of carbomer) was stirred, triethanolamine was added and then stirred slowly until a gel mass was formed. The results of the rosella flower extraction were mixed with glycerin and nipagin and then propylene glycol was dissolved in distilled water. The mixture obtained is added to the gel mass and then stirred until homogeneous. (Table-1)

Ingredients	Units	Formula				
		F1	F2	F3	F4	F5
Roselle ethanol extract	gram	5	10	15	20	30
Carbomer	gram	2	2	2	2	2
Propylene glycol	gram	5	5	5	5	5
Glycerin	ml	15	15	15	15	15
Triethanolamie	ml	12	12	12	12	12
Methyl paraben	gram	100	100	100	100	100
Aquadest	gram	100	100	100	100	100

**Table 1: Gel formula with a carbomer base.**

#### 4. Bacterial culture

The streptococcal bacteria used in this study were pure bacteria obtained from the laboratory of the Department of Anafarma, Poltekkes, Ministry of Health, Jakarta II. Bacteria were grown in blood agar and then incubated for 24 hours at 37°C. The best colonies from the plantings were taken and planted in BHI, incubated again for 5 hours at 37°C. Then serial dilution was carried out up to 108 CFU/ml. Microorganism growth (turbidity was matched with Brown III standard, 108 CFU/ml, then smeared on the surface of the agar media (a total of 15 petri dishes) evenly. The next step was making wells with a diameter of 5mm.

The research process was carried out on rosella flower extract with concentrations of 5%, 10%, 15%, 20%, 30% and as a control Amoxicillin. Each 10 ml was taken and then dropped into each well. Methods of Execution The experiment used a Single Dish, using 30 petri dishes of agar, then incubated in an incubator for 24 hours at a temperature of 37°C.

The data was obtained from the measurement of the diameter of the inhibition zone around the well. Measurements were made using a caliper with the aid of a microscope. The measurement method is: by taking two mutually perpendicular lines through the center point of the wellbore, while the third line is taken between the two lines by forming an angle of 45 degrees. Measurements were carried out 3 times at different places.

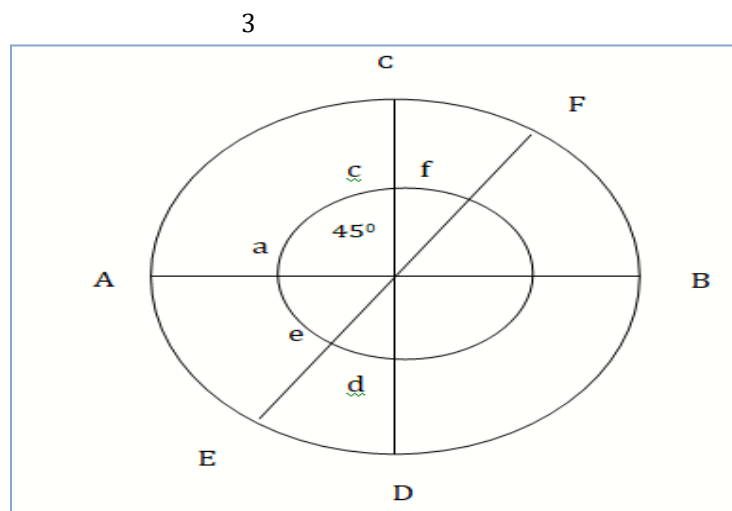
Measurements were made using the diameter of the resistance area (A-B) reduced by the diameter of the borehole (a-b) the results were divided by two, then measurement I was obtained. The measurement data II was obtained from the measurement of the diameter of the resistance area perpendicular to the measurement I (C-D) minus the diameter of the borehole (c-d) then the result is divided by two. Measurement data III is obtained from the diameter of the inhibition area at an angle of 45 degrees (E-F) minus the diameter of the borehole (e-f) then the result is divided by two. Measuring data I, II, III are taken on average so that the obstacle area is obtained.

Measurement I =  $(AB-ab) : 2$

Measurement II =  $(CD-cd) : 2$

Measurement III =  $(EF-ef) : 2$

Area of resistance =  $\frac{\text{Measurement I} + \text{Measurement II} + \text{Measurement III}}{3}$



**Figure 1: Measuring the Diameter of the Barrier Zone**

## Result

The concentration of 5% rosella flower extract in the form of a gel has shown an inhibition in the growth zone of *Streptococcus mutans* bacteria. This can be known by comparing the average results of the mutans streptococcal antibacterial test with positive and negative controls. The results of the measurement of the average inhibition of rosella flower extract in the form of the gel were the largest concentration of 30% at 14.4 mm while the smallest was the concentration of 5% at 10.8 mm. (**Table-2**)

No	Concentration	Inhibition zone diameter (mm)/repetition					
		I	II	III	IV	V	Mean
1	30%	14.0	14.6	14.3	13.9	15	14.4
2	20%	13.5	13.7	13.5	12.8	13.8	13.5
3	15%	13.0	12.9	12.7	12.5	12.9	12.8
4	10%	12.4	12.5	12.9	12.0	12.6	12.5
5	5%	11.0	10.7	10.9	11.0	10.3	10.8
6	K + (amoxicillin)	36.3	33.3	34.3	30	34.3	33.6
7	K - (blank disc)	6	6	6	6	6	6
8	Disc diameter	6	6	6	6	6	6

**Table 2: Distribution of the average inhibition of *Streptococcus mutans* bacteria on roselle flower extract in the form of a gel.**

The concentration of liquid form in Rosella extract 5% has not shown any inhibition in the growth zone of *Streptococcus mutans* bacteria and there is only inhibition in the zone of bacterial growth at a concentration of 10%. This can be known by comparing the average results of the Streptococcal Mutans Antibacterial test with the positive control and negative control. The results of the measurement of the average inhibition of roselle extract in liquid form were the highest concentration of 30% at 10.7 mm, while the lowest concentration was 5% at 6 mm. (**Table-3**)

No	Concentration	Inhibition zone diameter (mm)/repetition					
		I	II	III	IV	V	Mean
1	30%	10.0	11.0	10.8	10.4	11.4	10.7
2	20%	8.4	8.7	8.5	8.0	9.8	8.7
3	15%	7.3	7.5	7.6	7.5	7.3	7.4
4	10%	7.0	6.8	7.1	6.3	6.7	6.8
5	5%	6	6	6	6	6	6
6	K + (amoxicillin)	34	34	33.4	35.2	34	34.1

7	K - (blank disc)	6	6	6	6	6	6
8	Disc diameter	6	6	6	6	6	6

**Table 3: Distribution of the average inhibition of Streptococcus mutans bacteria on roselle flower extract in the form of a liquid.**

The results of the normality test of roselle flower extract data in the form of gel and liquid were normally distributed. It is known that based on the p-value > 0.05, the statistical analysis in this study can be continued with parametric tests. (Table-4)

No	Roselle flower extract	Concentration				
		30%	20%	15%	10%	5%
1	Gel	0,888	0,607	0,789	0,986	0,893
2	Liquid	1,000	0,801	0,930	0,989	1,000

\* Shapiro-Wilk

**Table 4: Test the normality of data on the inhibition of Streptococcus mutans bacteria on roselle flower extract in gel and liquid form.**

The results of the data effectiveness test showed that the p-value of the roselle extract in the gel form was 0.000 (p<0.05), meaning that the roselle extract in the gel form was effective in inhibiting the growth of the bacterium Streptococcus mutans. The p-value of the liquid roselle extract was 0.003 (p< 0.05). The liquid roselle extract was effective in inhibiting the growth of the bacteria Streptococcus mutans. (Table-5)

No	Roselle flower extract	Concentration					p-value
		Mean±SD (30%)	Mean±SD (20%)	Mean±SD (15%)	Mean±SD (10%)	Mean±SD (5%)	
1	Gel	14.76±1.114	13.46±0.391	12.80±0.200	12.48±0.327	10.78±0.295	0.000
2	Liquid	10.72±0.540	8.680±0.676	7.480±0.178	6.780±0.311	6.000±0.000	0.003

\*Repeated Measure Anova

**Table 5: Test the effectiveness of the inhibition of Streptococcus mutans bacteria in the gel and liquid groups.**

The results of the difference test showed that the p-value between the gel and liquid rosella flower extract at 30% concentration was 0.204 (p>0.05), 20% concentration was 0.427 (p>0.05), 15% concentration was 0.771 (p >0.05), and a 10% concentration of 1.000(p>0.05). This means that the gel and liquid roselle flower extract at concentrations of 30%, 20%, 15%, 10% are equally effective at inhibiting the growth of Streptococcus mutans bacteria. Meanwhile, the p-value between gel and liquid roselle flower extract at a



concentration of 5% was 0.012 ( $p < 0.05$ ), meaning that gelled roselle flower extract was more effective in inhibiting the growth of *Streptococcus mutans* bacteria than in liquid form. (Table-6)

Concentration	Roselle flower extract	Mean $\pm$ SD	p-value
30%	Gel	14.76 $\pm$ 1.114	0.204
	Liquid	10.72 $\pm$ 0.540	
20%	Gel	13.46 $\pm$ 0.676	0.427
	Liquid	6.680 $\pm$ 0.676	
15%	Gel	12.80 $\pm$ 0.200	0.771
	Liquid	7.480 $\pm$ 0.178	
10%	Gel	12.74 $\pm$ 0.327	1.000
	Liquid	6.780 $\pm$ 0.311	
5%	Gel	10.78 $\pm$ 0.295	0.012
	Liquid	6.000 $\pm$ 0.000	

\* Independent Sampel Test

**Table 6: Different test of *Streptococcus mutans* inhibition in the gel and liquid groups**

## Discussion

Rosella plants are shrubs that are easy to grow and flower throughout the year, Rosella flowers have various natural ingredients that are nutritious for health. The results of the determination of rosella plants by Herbarium Bogoriense, Division of the BRIN Cibinong Biological Research Center, the rosella plants studied were *Hibiscus sabdariffa* L. belonging to the Malvaceae tribe. Research on the effectiveness of rosella flower extract in the form of gel and liquid preparations in inhibiting the growth of *Streptococcus mutans* bacteria aims to determine and compare between gel and liquid preparations in inhibiting the growth zone of *Streptococcus mutans* bacteria.

The test of Rosella flower extract against the growth of *Streptococcus mutans* bacteria was known from the distribution of the average inhibitory power that was seen at a concentration of 5% of 10.8 mm, 10% of 12.5 mm, 15% of 12.8 mm, 20% of 13.5 mm and 30% by 14.4 mm in the gel preparation. Meanwhile, the average distribution of new liquid preparations showed that there was an inhibitory power of bacterial growth at concentrations ranging from 10% to 6.8 mm, 15% to 7.4 mm, 20% to 8.7 mm and 30% to 10.7 mm. The average inhibitory power of Amoxicillin positive control was 33.6 mm. it means that it can be seen that the average gel preparation has a larger zone of inhibition than the liquid preparation at each concentration.

The results of the paired data effectiveness test using Repeated Measure Anova showed that the p-value of the gelled rosella extract was 0.000 ( $p\text{-value} < 0.05$ ), meaning that the gelled rosella extract was effective in inhibiting the growth of *Streptococcus mutans* bacteria. The p-value of the liquid rosella extract was 0.003 ( $p\text{-value} < 0.05$ ), meaning that the liquid rosella extract was effective in inhibiting the growth of *Streptococcus mutans* bacteria.

The results of the different test using independent samples test with the T Test showed that the p-value between gel and liquid rosella flower extract at a concentration of 30% was 0.024 ( $p\text{-value} > 0.05$ ), 20% concentration was 0.427 ( $p\text{-value} > 0.05$ ), the 15% concentration was 0.771 ( $p > 0.05$ ), and the 10% concentration was 1,000 ( $p\text{-value} > 0.05$ ). This means that the gel and liquid rosella flower extracts at concentrations of 30%, 20%, 15%, 10% were equally effective at inhibiting the growth of *Streptococcus*



mutans bacteria. Meanwhile, the p-value between gel and liquid rosella flower extract at a concentration of 5% was 0.012 ( $p < 0.05$ ), meaning that the gel formulation was more effective in inhibiting the growth of *Streptococcus mutans* bacteria.

Research on the antioxidant content of rosella proving that three rosella buds were ground to a powder weighing 1.5 grams, then added 200 ml of water and put into a spectrophotometer. The antioxidant content of rosella is higher than that of cat's whiskers, which is 1.7 mmol/prolox. Making rosella extract by drying the flower petals at a temperature of 50°C for 36 hours. Furthermore, 3 grams of dried rosella was diluted with 300 ml of water, then put into a spectrophotometer. As a result, rosella is proven to contain 24% antioxidants and 51% anthocyanins. With the presence of antioxidants, free radicals that can damage the cell nucleus can be removed. Anthocyanin substances play a role in protecting cell damage from excess ultraviolet rays that are absorbed by the body.<sup>15,16</sup>

The results of this study can be developed to make gel and liquid preparations with more precise formulations so that the contents and properties can be utilized for health in general and for dental and oral health in particular.

### Conclusion

Based on the results of the study, it can be concluded that there is rosella flower extract was effective in inhibiting the growth of *streptococcus mutans* bacteria and rosella flower extract in gel form was greater than liquid in inhibiting the growth of *streptococcus mutans* bacteria.

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