

Inhibition Strength of Rosella (*Hibiscus sabdariffa L.*) Boiled Water on *Salmonella typhi* in vitro

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Abstract: Rosella (*Hibiscus sabdariffa L.*) is a plant that can use as herbal medicine. Roselle calyx contains active compounds flavonoids, saponins, and tannins. These compounds can use as an antibacterial against *Salmonella typhi*. This study aims to determine the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration and the effect of rosella cooking water concentration on *Salmonella typhi* in vitro. Experimental research with posttest only controls group design through dilution method. The results of the Minimum Inhibitory Concentration (MIC) study showed clarity at concentrations of 60%, 70%, 80%, 90%, and 100%. Minimum Bactericidal Concentration (MBC) results obtained the number of colonies at a concentration of 60% by one colony, at a level of 70%, 80%, 90%, and 100% showed no colony growth. Based on the results of the study concluded that there was an influence of rosella cooking water concentration on the growth of *Salmonella typhi* in vitro.

Keywords: rosella (*Hibiscus sabdariffa L.*); *Salmonella typhi*

INTRODUCTION

Rosella (*Hibiscus sabdariffa L.*) is a plant native to tropical Africa, including the Hibiscus species and the Malvaceae Family. The community commonly uses this plant cultivated in many countries such as Indonesia, India, Malaysia, Egypt and Sudan¹. The rosella calyx as a fresh beverage ingredient in the form of syrup, tea, jam and other types of herbal drinks². Rosella calyx tea was seen to be beneficial for health, namely as an antioxidant³. Roselle calyx contains active anthocyanin compounds, such as cyanidin-3-sambubioside, cyanidin-3-glucoside, and delphinidin-3-glucoside⁴ flavonoids, saponins, and tannins⁵. These compounds can use as antibacterial. Like the propolis *Trigona sp* which is antibacterial⁶.

Extracts from *Hibiscus sabdariffa L.* roselle calyx have antimicrobial effects on various pathogenic microorganisms^{7,8,9,10,11,12}, including *Salmonella*¹³. Based on research by Rostinawati (2009), the results showed that the minimum inhibitory concentration of the rosella flower test plant (*Hibiscus sabdariffa L.*) was 0.20 g / ml against *Escherichia coli* and *Salmonella typhi*¹⁴. The results of other studies stated that the dilution of rosella stew at a concentration of 80%-100% is sensitive which indicates the diameter of the zone of growth inhibition of *Escherichia coli* bacteria 15-16 mm⁵.

The antimicrobial effect of Rosella (*Hibiscus sabdariffa L.*) extracts against *Salmonella* has been proven, but the inhibitory properties of the Rosella (*Hibiscus sabdariffa L.*) starch water in the same bacteria are unknown. The purpose of this study was to determine the effect of rosella cooking water concentration (*Hibiscus sabdariffa L.*) on the growth of *Salmonella typhi* in vitro.

MATERIALS AND METHODS

This type of research is an experiment with a Posttest Only Control Group Design, namely by examining the inhibitory and killing power of boiled water of rosella petals (*Hibiscus sabdariffa L.*) at concentrations of 60%, 70%, 80%, 90%, and 100%. It then compared with the negative control group, positive control, and control boiled water by repeating four times.

The materials used are fresh rosella plants in the Kuala Kapuas region of Central Kalimantan. The plant part used is sweet, dark red petals. The independent variable in this study was the concentration of boiled water of rosella calyx (*Hibiscus sabdariffa L.*). The dependent variable in this study was the level of turbidity produced in Tryptic Soy Broth (TSB) media to see the Minimum Inhibitory Concentration (MIC). And the number of *Salmonella typhi* bacterial colonies on nutrient media to see the Minimum Bactericidal Concentration (MBC). Rosella plant determination test conducted at the Faculty of Mathematics and Natural Sciences University of Lambung Mangkurat Banjarmasin Indonesia.

We were making rosella boiled water (*Hibiscus sabdariffa L.*). Clean rosella from pests weighed 200 grams, washed thoroughly with running water, then put in a sterile glass beaker and added with 100 ml of sterile distilled water (200% concentration). Cover the beaker glass using aluminum foil. Then the mixture is heated 90°C on a hot plate for 15 minutes. After boiling water is added to 100 ml, allowed to stand at room temperature until cool, and filtered with sterile gauze in a measuring flask. Subsequently diluted again so as to obtain a concentration of 120%, 140%, 160 %, 180%, 200%.

The bacterial suspension was obtained by *Salmonella typhi* culture for 4–8 hours at 37°C. Determination of MIC by adding 1 mL of the solution of various concentrations with 1 mL of bacterial suspension so that the final level of the solution becomes half the initial concentration of 60%, 70%, 80%, 90%, and 100%. The positive control used chloramphenicol solution with the addition of a bacterial suspension. The only negative control was *Salmonella typhi* bacterial suspension in the TSB. Repetition did four times. Incubate 37 ° C for 24 hours. Tubes that contain the lowest levels but are still able to inhibit bacterial growth marked by clear colored solutions expressed as MIC values¹⁵.

Determination of MBC by taking a MIC suspension at each concentration of 50 ul and then spread on a Nutrient plate to do four repetitions. Incubated 24 hours at 37 ° C. Colonies grown on SDA were counted¹⁶.

RESULTS AND DISCUSSION

The antibacterial test of rosella cooking water on the growth of *Salmonella typhi* showed the level of clarity in the determination of Minimum Inhibitory Concentration (MIC), which can see in Table 1.

Table 1. Results of MIC of Rosella Stewed Water on Growth *Salmonella typhi*

Repetition	Concentration of Rosella Stewed Water					Control		
	60%	70%	80%	90%	100%	Negative Control	Positive Control	Rosella Control
1	Clear	Clear	Clear	Clear	Clear	Turbid	Clear	Clear
2	Clear	Clear	Clear	Clear	Clear	Turbid	Clear	Clear
3	Clear	Clear	Clear	Clear	Clear	Turbid	Clear	Clear
4	Clear	Clear	Clear	Clear	Clear	Turbid	Clear	Clear
Conclusion of results	Clear	Clear	Clear	Clear	Clear	Turbid	Clear	Clear

Based on the determination of the Minimum Inhibitory Concentration (MIC), it found that the growth of the colony occurs at a concentration of 60% with the number of colonies as much as 1 colony and there is no growth at other levels, for more details, it can see in the details in Table 2.

Table 2. Results of MBC of Rosella Stewed Water on Growth *Salmonella typhi*

Repetition	Concentration of Rosella Stewed Water					Control		
	60%	70%	80%	90%	100%	Negative Control	Positive Control	Rosella Control
1	2	0	0	0	0	4.544	0	0
2	1	0	0	0	0	4.624	0	0
3	1	0	0	0	0	4.064	0	0
4	1	0	0	0	0	4.720	0	0
Average	1	0	0	0	0	4.488	0	0

Based on Table 2, generally known, bacteria do not grow in the addition of rosella boiled water (*Hibiscus sabdariffa L.*) concentration of 70% - 100%. So that the Minimum Bactericidal Concentration (MBC) of rosella boiled water (*Hibiscus sabdariffa L.*) against *Salmonella typhi* is at the concentration of 70%.

Calculation of the number of bacterial colonies done in two ways. If the colonies grow a little and can still count manually, then the number of colonies is counted manually. If the colonies grow a lot, then the number of colonies is calculated using the Colony Counter. Furthermore, the results of the calculation of the number of colonies in Table 2 are analyzed to see the effect of rosella cooking water (*Hibiscus sabdariffa L.*) on *Salmonella typhi*.

The first test conducted is a normality test to calculate the distribution of data obtained in the study. Normality test results show the results obtained in the form of $p < 0.05$, which means that the data distribution is not normal. An alternative test use is the

Kruskal-Wallis test (Nonparametric Test). Based on the Kruskal-Wallis test, it found that the value of $p < 0.05$ is 0,000. Shows that there are differences in the average cumulative number of *Salmonella typhi* bacterial colonies in each treatment, namely from bacterial control and the provision of rosella cooking water at each concentration. Then the Mann-Whitney test was performed to determine the difference in the influence of rosella boiled water (*Hibiscus sabdariffa L.*) between the level with other strengths and the concentration with the control of *Salmonella typhi* bacteria.

Table 3. Results of Uji *Mann-Whitney* of Rosella Stewed Water on Growth *Salmonella typhi*

	Concentration 1 (60%)	Concentration 2 (70%)	Concentration 3 (80%)	Concentration 4 (90%)	Concentration 5 (100%)
Negative Control	0,018	0,014	0,014	0,014	0,014

There was a significant difference if $p < 0.05$. Based on table 3, it that the most significant influence is found on the concentration of Rosella cooking water 70% (significance 0.014) when compared with bacterial control. Significance value is getting closer to 0.000 shows that the difference caused is getting stronger.

Based on the results of this study, Rosella boiled water has an antibacterial effect, both inhibiting and killing against *Salmonella typhi* bacteria. The minimum concentration of Rosella cooking water that can still inhibit bacteria is at a level of 60%, whereas at a concentration of 70% Rosella cooking water has a killing effect (Table 1.2). The results of the study marked by the occurrence of clarity in the tube dilution test TSB media and a decrease in the number of *Salmonella typhi* colonies growing on the surface of the Nutrient agar media. In bacterial control, the average number of germs growing was 4,488 colonies. At a concentration of 60%, Rosella boiled water of 1 colony, at a level of 70%, 80%, 90% and 100% of 0 colonies. Whereas in the control sample that only contained Rosella cooking water, no bacterial colonies were found to grow.

Research shows that rosella cooking water has antibacterial power with *Salmonella typhi* test bacteria. Antibacterial testing using the tube dilution method showed the results of the Minimum Inhibitory Concentration (MIC) at a concentration of 60%. This statement is supported by research by Riyaniarti and Susilo (2015), who stated that rosella cooking water could inhibit the growth of gram-negative bacteria with *Escherichia coli* test bacteria at a concentration of 80%. Other studies have shown *Hibiscus sabdariffa L.* Has antimicrobial activity against human pathogenic bacteria¹⁷.

Based on the results of the Kruskal-Wallis statistical test, a p-value of < 0.05 is 0.000 (Table 3). Shows that there is a difference in the average cumulative number of *Salmonella typhi* bacterial colonies in each treatment, namely from bacterial control and rosella cooking water treatment at each concentration. The ability of rosella to kill *Salmonella typhi* bacteria according to research conducted by Rostinawati (2009) from the phytochemical screening of rosella flower test plants (*Hibiscus sabdariffa L.*) detected the presence of alkaloids, flavonoids, saponins and tannins which have

antibacterial activity. The antimicrobial activity of Rosella in several studies has also linked to protocateic acid and anthocyanins^{7,8,9,10,18}.

CONCLUSION

Minimum Inhibitory Concentration of Rosella (*Hibiscus sabdariffa* L.) cooking water against *Salmonella typhi* occurs at a concentration of 60%. Minimum Bactericidal Concentration of Rosella (*Hibiscus sabdariffa* L.) cooking water against *Salmonella typhi* occurs at a level of 70%. There is an influence of the concentration of rosella (*Hibiscus sabdariffa* L.) boiled water on *Salmonella typhi* in vitro.

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