

Research Report

THE ANTIBACTERIAL EFFECT OF ROSELLE (*Hibiscus sabdariffa*) EXTRACT AGAINST *Staphylococcus epidermidis* IN VITRO

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ABSTRACT

*Infection of *Staphylococcus epidermidis* is still a common problem in many hospitals. Factor determining biofilm formation makes it harder for antibiotics to cure the infection. Roselle (*Hibiscus sabdariffa*), a well known traditional medicine plant, is a potential candidate as a drug against infectious disease. The purpose of this research is to investigate the antibacterial effect of ethanol extract from Roselle (*Hibiscus sabdariffa*) calyx against the growth of *Staphylococcus epidermidis*. Assessment for antibacterial effect is performed using broth diffusion method. The extract is made by maceration of the calyx of Roselle in 96% ethanol. Extracts with concentration of 125, 62.5, 31.25, 15.63, 7.81, 3.90, 1.95, 0.97, 0.48, 0.24 mg/mL are added into separated Mueller-Hinton broths (MHB), which have already been inoculated by *Staphylococcus epidermidis*. As for bacterial growth control, we used MHB with bacterial inoculation, while sterility control we used mixture of extract and MHB. Then from each broth, the solutions are added into separated nutrition agar plates. Replications are done three times. Clarity and bacterial growth are observed after 24 hours of incubation. However, clarity cannot be observed in 36 broth, but bacterial growth is observed on the plate for concentration 0.97, 0.48, and 0.24 mg/mL. Therefore Minimum inhibitory concentration (MIC) cannot be determined because the extract's color interfere the observation. While minimum bactericidal concentration (MBC), the last concentration before the concentration where the bacteria are still viable, is 1.95 mg/mL. Based on the result of the research, the Roselle calyx ethanol extract (*Hibiscus sabdariffa*) through dilution method with a concentration of 1.95 mg / mL can kill *Staphylococcus epidermidis* and in order to find MIC in collored and turbid solution (before being incubated in incubator), we can consider using agar dilution method or microdilution method.*

Keywords: *Hibiscus sabdariffa*, antibacterial, *Staphylococcus epidermidis*, biofilm, flavonoids

ABSTRAK

*Infeksi *Staphylococcus epidermidis* masih merupakan masalah umum yang ditemukan di banyak rumah sakit. Kemampuan bakteri untuk membuat biofilm mempersulit antibiotik untuk menyembuhkan infeksi. Rosella (*Hibiscus sabdariffa*), tanaman obat tradisional yang umum beredar di masyarakat, adalah bahan yang berpotensi untuk dikembangkan menjadi obat untuk mengatasi infeksi. Tujuan dari penelitian ini adalah untuk mengetahui efek antibakteri dari ekstrak etanol kelopak Rosella (*Hibiscus sabdariffa*) terhadap pertumbuhan *Staphylococcus epidermidis*. Penelitian ini merupakan penelitian eksperimental laboratorium dengan metode dilusi. Ekstrak Rosella dibuat dengan cara maserasi dari tampuk Rosella dengan menggunakan etanol 96%. Kemudian dilakukan pengenceran sebanyak 10 kali didalam 10 tabung. Konsentrasi yang didapatkan adalah 125, 62.5, 31.25, 15.63, 7.81, 3.90, 1.95, 0.97, 0.48, 0.24 mg/mL. Masing-masing tabung sudah berisi bakteri. Sebagai kontrol tumbuh bakteri digunakan campuran bakteri dengan Mueller-Hinton broth dan kontrol sterilitas menggunakan cairan ekstrak dengan Mueller-Hinton broth. Kemudian dari ke-12 tabung, dilakukan streaking pada media nutrient agar plate untuk melihat pertumbuhan dari bakteri. Replikasi percobaan dilakukan sebanyak 3 kali hasil percobaan diamati setelah inkubasi selama 24 jam. Hasil penelitian yang didapat, dari 36 tabung tidak dapat diamati kejernihan dari tabung. Hal ini disebabkan warna dari ekstrak mengganggu dari kejernihan pengamatan sehingga tidak dapat ditentukan nilai dari konsentrasi hambat minimal (KHM). Kemudian dari nutrient agar plate, didapatkan pertumbuhan bakteri pada*

konsentrasi 0.97, 0.48, dan 0.24 mg/mL. hal ini berarti bahwa konsentrasi bunuh minimal (KBM) dari ekstrak Rosella adalah 1.95 mg/mL. Berdasarkan hasil penelitian yang dilakukan, ekstrak tampuk Rosella dengan menggunakan metode dilusi dapat membunuh bakteri *Staphylococcus epidermidis* pada konsentrasi 1.95 mg / mL dan untuk memperoleh hasil KHM pada larutan yang berwarna dan keruh (sebelum diinkubasi dalam inkubator). dapat dipertimbangkan untuk menggunakan metode dilusi agar atau mikrodilusi. Sehingga Rosella memiliki efek antibakterial dan memiliki potensi yang besar sebagai bahan antimikroba.

Kata kunci: *Hibiscus sabdariffa*, antibacterial, *Staphylococcus epidermidis*, biofilm, flavonoids

INTRODUCTION

Bacterium is the microorganism that is most frequently found in human body. This microorganism often causes infection and medical problems. One of them is *Staphylococcus epidermidis*. Normally, this bacterium is present in healthy people and doesn't cause infectious disease, however in certain condition like immunodeficiency syndrome, it can cause infectious diseases.¹ Because of this condition, *Staphylococcus epidermidis* is a common cause of nosocomial infections. As many as 40-90% of nosocomial infections associated with hospital tools are caused by this bacterium.² This increases the patient's health expenditure and duration of staying in hospital. In America, 50-70% of the 16,000 cases of bacteremia by catheter infection in ICU are caused by *S. epidermidis* with an additional cost of 37.000-39.000 US\$ for each person.³ Besides the high incidence rate, many strains of *Staphylococcus* have antibiotics resistance like methicillin and vancomycin. This resistance is also associated with the bacteria's ability to make biofilms.⁴ Biofilm is an ability possessed by a certain kind of bacteria to bind and create a complex structure which is formed by its colonization. It has the ability to allow bacteria to develop resistance to host immune response and antibiotics.⁵ Therefore, the discovery of treatments to infectious diseases, in particular caused by *S. epidermidis* is very important.

In addition to drug development, the use of medical plants as natural antimicrobial agents is gaining popularity. Roselle (*Hibiscus sabdariffa*) is a tropical and sub-tropical plant with a potential candidate in herbal medicine. It is commonly used to make form drink and pickle and it is used in folk medicine infor treatment of hypertension, liver disease and fever.⁶ Several studies have been conducted on Rosella and have shown various benefits for medical purpose. A research was conducted by Majorie *et al.* Bioactive substances in Hibiscus like alkaloids, flavonoids, phenolics and biterpenoids) may have antibacterial effect against *Esherichia coli*.⁷ Anthocyanin and protocatechuic acid compounds also isolated from dried flower of Hibiscus sabdariffa demonstrate protective effects against oxidative agents.⁶ Moreover, a research also conducted found that the protocatechuic acid also inhibited the growth of methicillin-resistant *S. aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Acinetobacter baumanniliu*, *tsao*, *yin*).⁸

With the increase in *Staphylococcus* resistance and Roselle medical potential, we need further research regarding the antibacterial effect of this plant. In this study, researcher aims to investigate the antibacterial effect of Roselle extract on the growth of *Staphylococcus epidermidis* in vitro.

MATERIAL AND METHOD

Plant Material Extraction

The flowers of *Hibiscus sabdariffa* were purchased from UPT Materia Medica in Batu small town, east java. The plant materials were taxonomically identified by a botanist at the same location. Calyces of the plant were separated and ground to a fine powder. About 250 g dried powder was taken and soaked with 96% ethanol. The wet powder is put in a jar and as much as 1500 mL 96% ethanol was poured into the jar. The jar was then closed tightly for 24 hours and placed on the shaker with 50 rpm. After that the suspension was filtered and was placed into erlenmeyer. The sediment from the filtration was re-maceration with the same technique (1500 mL 96% ethanol). The ethanolic extract was evaporated with rotary evaporator for 2 hours and water bath for 1 hour. The final result from the extraction was 77 mL extract with concentration 250 g/mL.

Antibacterial Assay

Prior to the experiment, a colony of *S. epidermidis* was subcultured in Mueller Hinton Agar (MHA) and incubated for 24 hours at 37 °C. The bacteria were then adjusted by adding normal saline to be equivalent to 0.5 McFarland standard which comprised 1.0×10^8 .

Susceptibility Testing Procedure

The experiment was repeated 3x. The extract was dispensed in 1 mL volume in each sterile tube with decreasing concentration starting from 125 g/mL. Each tube was then inoculated by 1 mL volume of diluted *S. epidermidis*. The growth control tube consists of 1 mL inoculum and extract free medium while the sterility control contains 1 ml extract and 1 ml medium. All tubes were incubated at 37 °C and MICs were read after 24 hours of incubation.

RESULT

A total of 30 tubes were tested for MIC. The result of the MIC is shown in Table 1.

Overall, by using macrodilution method the MIC couldn't be determined. This result happens because the extract's red color and turbidity interfere with the observation and the assessment. The same condition could also be seen in sterility control tube because of that it couldn't be used as a comparison to determine sterility. so in order to determine the extract's efficacy, each of the 30 tubes was streaked into nutrient agar plate to determine MBC value which was shown in Table 2.

From the experiment, it can be determined that the MBC of Roselle extract against *S. epidermidis* is 1.95 mg/mL (1.56%).

DISCUSSION

Roselle is a great plant to be used for medical purpose. First, it is easily grown in tropical country like in Indonesia and has many properties. The time required to grow is around 4 to 8 months with the lowest temperature 20°C, 13 hours lighting, and 130 to 250 mm of rainfall for each month.⁹ With these condition, people can easily get the plant and cultivate it. Second, it is known to have many good effects. Roselle has antimicrobial, anti-parasite, anti-cancer, anti-pyretic, anti-inflammation, anti-oxidant, nephro-protective, hepato-protective, diuretic, anti-cholesterol, antidiabetic, and antihypertensive.^{6, 10-11}

From the result, the MIC in the experiment couldn't be identified because of the extract's red colour and turbidity. To find out the result of the MIC, extract Roselle can be tested with another dilution method. In Nigeria, research was conducted by Mary¹². She did MIC testing by agar dilution method. She examined the antimicrobial effect of Roselle against *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia marseilles*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, and *Pseudomonas fluorescense* with MIC 0.30 ± 0.2 - 1.30 ± 0.2 mg/mL. A similar research was conducted by Sulistyani¹³ and her research group with microdilution method. They tested antimicrobial activity against mouth pathological bacteria that could make biofilm. These bacteria were *Streptococcus mutans*, *Streptococcus sanguinis*, *Lactobacillus casei*, *Actinomyces naeslundii*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Prevotella intermedia* with MIC and MBC 7.2 mg/mL to 28.8 mg/mL and 14.4 to >57.6 mg/mL. Interestingly, Roselle extract also has the ability to inhibit biofilm formation on the concentration of the MIC.¹³ The formation of biofilm is also found in *S. epidermidis*.⁴

The overall mechanism how Roselle extract has antibacterial effects is still not completely comprehended. In USA, Marjorie, Janak, Jacqueline, Shurrita, and Leonardo were conducted a research about the antimicrobial activity of *Hibiscus sabdariffa* against *E. coli*. By using disk diffusion method, they concluded gain conclusion that all concentration (10%, 5%, and 2.5%) of *H. sabdariffa* could

Table 1. Roselle extract's MIC

	Extract Concentration										G+	S-	
	100% (1)	50% (2)	25% (3)	12.5% (4)	6.25% (5)	3.12% (6)	1.56% (7)	0.78% (8)	0.39% (9)	0.19% (10)			
1	X	X	X	X	X	X	X	X	X	X	X	X	X
2	X	X	X	X	X	X	X	X	X	X	X	X	X
3	X	X	X	X	X	X	X	X	X	X	X	X	X

G+: Growth control

S-: Sterility control

X: Can't be assessed

Table 2. Roselle extract's MBC

	Extract Concentration										G+	S-	
	100% (1)	50% (2)	25% (3)	12.5% (4)	6.25% (5)	3.12% (6)	1.56% (7)	0.78% (8)	0.39% (9)	0.19% (10)			
1	-	-	-	-	-	-	-	+	+	+	+	+	-
2	-	-	-	-	-	-	-	+	+	+	+	+	-
3	-	-	-	-	-	-	-	+	+	+	+	+	-

G+: Growth control

S-: Sterility control

+: Viable bacteria

-: No viable bacteria

inhibit *E. coli* activity in from food, veterinary, and clinical samples and showed that the most effective concentration was at 10%, whereas the least effective concentration was at 2.5%. They were stated that the antimicrobial effect of *H. sabdariffa* might come from its flavonoids chemical. The structure of flavonoids have the ability to form combined complex with bacterial walls. Besides that, the number of hydroxyl groups present on the phenolic ring helps the antimicrobial activity of the extract. Due to the increase of hydroxyl group, the hydroxylation would accelerate and cause the increase of antimicrobial activity.⁷ Issam and Ahmed alsowere stated a similar discussion that phenolic compounds including flavonoids and cyaniding contribute to antimicrobial activity. They added that flavonoid with its phenolic chain could decreases iron level and increases hydrogen level, which deactivates bacterial enzymes.¹⁴ Moreover Sulistyani *et al.* reported that Flavonoids are also thought to have the ability to inhibit the formation of bacterial biofilms. This capability is possible because the phenolic group in the extract capable to bind strongly to proteins and enzymes from the bacteria. This makes the bacteria unable to produce biofilms.¹³ This effect is important considering *S. epidermidis* and some gram-positive and gram-negative bacteria capable of producing biofilms.

Based on the research that has been conducted, Roselle calyx extract can be used as a alternative treatment for infections caused by *Staphylococcus epidermidis*.

CONCLUSION

The Roselle calyx ethanol extract (*Hibiscus sabdariffa*) through dilution method with a concentration of 1.95 mg / mL can kill *Staphylococcus epidermidis* and in order to find MIC in collored and turbid solution (before being incubated in incubator), we can consider using agar dilution methode or microdilution methode.

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