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Anti-bacterial activity of *rosella* flowers extract (*Hibiscus sabdariffa linn*) in inhibiting bacterial growth methicillin-resistant *Staphylococcus aureus*

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Abstract. Infections caused by bacteria *Methycillin Resistant Staphylococcus aureus* (MRSA) has become a worldwide health problem because of its increasing incidence. *Rosella* flower (*Hibiscus sabdariffa Linn*) has been known to have efficacy as an antibacterial. The purpose of this study is to determine the antibacterial activity of *rosella* flower extract in inhibiting the growth of MRSA. This study is an experimental research laboratory using *rosella* flower extract as a treatment, *vancomycin* as a positive control and distilled water as negative control. Fresh and dried *rosella* flowers crushed and then macerated with 96% ethanol for 24 hours. The filtrate obtained was concentrated by rotary evaporator at a temperature of 35 ° C-40 ° C and then diluted to a concentration of 12.5%, 25%, 50% and 75%. *Phytochemical* test is then performed. *S.aureus* bacteria isolated from clinical specimens in RSUDZA. Test of the antibacterial activity of *rosella* flower extract performed using Kirby Bauer disc diffusion method. Parameters on the disc were measured in millimeters MRSA. The data obtained were statistically analyzed and grouped into categories of Greenwood (1995). Phytochemical test results indicate the presence of *rosella* flower extract contains *flavonoids*, *tannins, saponins, triterpenoids* and *alkaloids*. Test results of the inhibition of *rosella* flower extract concentration of 12.5%, 25%, 50% and 75% of the growth of MRSA each inhibitory zone formed with an average diameter of 14.2 mm, 19.2 mm, 22.8 mm and 24.18 mm, whereas the positive control and negative control respectively of 19.8 mm and 5 mm. The results of data analysis showed that the ethanol extract of *rosella* flowers significant effect in inhibiting the growth of MRSA by the value of $p < 0.05$. Based on the criteria of Greenwood, *rosella* flower extract ethanol concentration of 50% and 75% belong to the category of strong inhibitory power in inhibiting the growth of MRSA, a concentration of 25% falls into the category of being and the concentration of 12.5% falls into the category of weak. The results can be concluded that *rosella* flower ethanol extract can inhibit the growth of MRSA.
Keywords: Rosela (*Hibiscus Sabdariffa* Linn.), MRSA, inhibition, antibacterial.

Introduction

Staphylococcus aureus is an important pathogen in humans. The presence of beta-lactamase enzymes in most *S. aureus* causes resistance to *penicillin (methycillin)* that are classified as *Methycillin Resistant Staphylococcus aureus (MRSA)*. Therefore the current MRSA infection has become a worldwide health problem. Antibiotic treatment option for infections caused by MRSA is *vancomycin*, however, lately known that the sensitivity of MRSA to *vancomycin* begin to decline (Murray *et al*, 1995; Salmenlinna, 2002; Katzung, 1997; Sudigdoadi, 2010). The failure in modern medicine against infectious diseases as described above, will encourage research to find alternative compounds that can be used to treat MRSA infections. The current study focused on the use of herbs for traditional medicine. This is because not only cheap and easy to obtain, but also plants used as traditional medicines have side much lower effects than chemical drugs (Sari, 2006).

Rosella flower (*Hibiscus sabdariffa Linn*) has been known to have antibacterial effect as it has content of secondary metabolites such as *glycosides, flavonoids, saponins, triterpenoids* and *alkaloids*. It also has been proven that *rosella* flower possess antibacterial activity against *Bacillus stearothermophilus, Micrococcus luteus, Staphylococcus aureus, Serratia mascences, Clostridium sporogenes, Escherichia coli, Klabsiella pneumoniae, Bacillus cereus* and *Pseudomonas fluorescence*. According Wibowo, Yuliana and Rimayanti (2009), *rosella* flower petals can prevent inflammation of the urinary tract and inhibit the growth of fungi or bacteria that causes high heat. Research on *rosella* flower antibacterial effect against MRSA so far has not been done. Therefore the purpose of this study is to determine the antibacterial activity of *rosella* flower extract in inhibiting the growth of bacteria MRSA.

Materials and Methods

Place and Time

The study was conducted from June to December 2011. Fresh *rosella* flowers obtained from Blang Bintang sub-district, Aceh Besar. To determine the taxonomic of the plants, a study was performed in the laboratory of the Department of Biological Science Herbarium. *Rosella* flowers extracting was performed in the Laboratory of Biological Chemistry Department Research

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FMIPA Unsyiah, bacteria and sampling re-identification conducted at the Laboratory of Microbiology RSUDZA. Test for *rosella* flower extracts for antibacterial activity against MRSA growth performed at the Laboratory of Microbiology Faculty of Medicine Unsyiah.

Research Design

This type of research is experimental research laboratories using completely randomized design (CRD) were divided into six groups: four experimental groups consisting of *rosella* calyx ethanol extract with a concentration of 12.5%, 25%, 50%, 75%, and 2 groups control, the positive control group given *vancomycin*, and negative controls given distilled water. Each group repeated 5 times.

Re-Identification of Bacteria

Bacteria were obtained from Clinical Microbiology Laboratory dr. Zainoel Abidin. The bacteria were collected from sputum of patients with pneumonia. Bacteria re-identification were performed by observing the macroscopic shape, surface, color and edge of the colony and the type of hemolysis, followed by Gram stain, catalase test and coagulase test. Determination of MRSA through antibiotic sensitivity test using oxacillin 1 microg on MHA media.

***Rosella* flower petals Extraction and Phytochemicals Test**

One kg fresh *rosella* flower petals were separated from flower seeds, then cleaned and dried. Dried *rosella* flower petals macerated for 1 × 24 hours with 96% ethanol, then concentrated with a rotary evaporator at 35 ° C-40 ° C until a viscous extract. This 100% concentration thick extract was then diluted to a concentration of 75%, 50%, 25% and 12.5%.

Phytochemical test conducted on fresh *rosella* flower petals and ethanol extract of *rosella* petals. The contents that examined were *alkaloids, steroids, terpenoids, saponins, flavonoids* and *tannins.*

Antibacterial Activity Test of Ethanol extracts of *Rosella* flower petals against MRSA

Antibacterial activity assays using Kirby Bauer disc diffusion method. The MRSA colonies were suspended in distilled water and then measured the density of bacteria divortex up to the equivalent to 109 bacteria per ml, using a spectrophotometer at a wavelength of 625 nm and the absorbance of 0.08 to 0.1. Suspension germs with a cotton swab dipped in sterile stick and scratched evenly on the surface of MHA media. Paper discs soaked with ethanol extract of *rosella* petals with various concentrations placed on the media. Those were also done to the positive control and negative control. The media were incubated at 37 ° C for 24 hours. Then formed bacterial growth inhibition zone was observed.

Results and Discussion

Results Acquisition *Rosella* Flowers

The extraction of 1 kg of fresh *rosella* petals gained as much as 100 grams of dried *rosella* flowers. From 100 grams of dried *rosella* flower obtained 8 ml viscous extract.

Phytochemicals Test Results

Ethanol extract of *Rosella* calyx of fresh *rosella* flower petals shows that it contains *flavonoids, tannins, saponins, triterpenoids* and *alkaloids.* These were shown with changing color and precipitation that occured in *phytochemical* test. While steroid compounds is not contained in the *rosella* flower petals, whether they are fresh or extracted with ethanol (Table 1).

Table 1. Phytochemical test results *rosella* flower petals

No	Phytochemical test	Fresh *rosella* flower petals	Ethanol extract of *rosella* flower petals	Result
1	Alkaloid			
	-Wagner	+	+	Color : brown
	-Dragendorf	+	+	Red precipitate
2	Flavanoid	+	+	Color : pink
3	Tanin	+	+	Color : black
4	Saponin	+	+	Stable foam
5	Triterpenoid	+	+	Red precipitate
6	Steroid	-	-	Color : Red

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Rostinawati (2008) reported that ethanol extract of *rosella* calyx contains *alkaloids, polifenolat, tannins, flavonoids, monoterpenoid, seskuiterpenoid, steroids* and *quinone* compounds, but does not contain *saponins* and *triterpenoids*. The diversity of the secondary metabolites of *rosella* flowers may be due to differences in the concentration of the solvent that is used during extraction, hydrolysis reactions due to heating during the extraction process so that the levels are too low and undetectable (Iriano, 2008), or because of environmental conditions and plant interactions between plants and their environment (Kutchan, 2001).

Re-Identification of MRSA Results

The observation of macroscopic growth of S. aureus on blood media showed the spherical shape of colony size of about 5-6 mm, the surface looks smooth, slightly elevated, and found cream-colored hemolysis. According to Murray *et al*. (1995) colonies of S. aureus were grown on blood agar media usually large in diameter 6-8 mm, looks smooth, slightly raised and shiny. In Gram staining, S. aureus looked purple and coccus-shaped indicating Gram-positive bacteria. Catalase test result is positive because air bubbles formed after peroxide spilled acids. This is because the staphylococci produce catalase enzyme that converts hydrogen peroxide into water and oxygen (Brooks, Butel and Morse, 2008). Coagulase test also gave positive results which is characterized by the formation of clots after spilled *Serobact Staph Latex* reagents, these results prove that the bacteria produce a protein that has been classified as coagulase *Staphylococcus aureus* species (Todar, 2008). Tests showed that *S. aureus* antibiotic resistance obtained was resistant to oxacillin. These results demonstrate that *S. aureus* tested is a MRSA bacteria.

Antibacterial Activity against MRSA *Rosella* Flower Extract Result

The test results of antibacterial activity of ethanol extract of *rosella* calyx 12.5%, 25%, 50% and 75% respectively produced inhibition zone with an average diameter of 14.2 mm, 19.2 mm, 22.8 mm and 24, 8 mm. Meanwhile, the average diameter of inhibition zone formed at the negative control and positive control, respectively 5 mm and 19.8 mm (Table 2 and Figure 1). These results indicate that the higher the concentration of *rosella* flower extracts were given, the greater the inhibition zone diameter were formed.

Table 2. Inhibition Zone Diameter *Rosella* flower extracts on growth of MRSA

Treatment	Inhibition zone diameter in each repeating (mm)					Total	Average
	I	II	III	IV	V		
P_0	5	5	5	5	5	5	5
P_1	14	14	14	14	15	71	14,2
P_2	19	19	19	20	19	96	19,2
P_3	22	24	22	23	23	114	22,8
P_4	24	26	23	26	25	124	24,8
P_5	19	20	19	20	21	99	19,8

Note: P0: distilled water; P1: *rosella* flower extract 12.5%, P2: *rosella* flower extract 25%; P3: *rosella* flower extract 50%, P4: *rosella* flower extract 75%; P5: Vancomycin

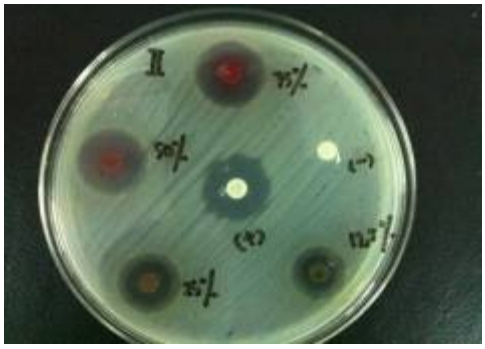

Figure 1. Inhibition Test Power Flower Extract Rosela against MRSA

The data analysis by Kruskal-Wallis test gives Asymptotic Significance values or p values of 0.000 (Asymp. Sig <0.05). This suggests that each treatment group gave significantly different results in inhibiting the growth of MRSA. Further test by Mann-Whitney method showed that there were significant differences in the treatment group P1, P3, and P4 compared to P5 where the value of p <0.05. While it is not significantly different than the P2 P5 (p> 0.05). These results indicate that the *rosella* flower extract concentration of 25% has antibacterial activity similar to vancomycin. According to Greenwood (1995), the inhibition of natural plant extracts virtually no power resistor if the diameter of the inhibition zone formed <10 mm, considered to be weak between 10 mm and 15 mm, moderate between 15 mm and 20 mm, strong if > 20 mm . Referring to the scale of the inhibition zone, *rosella* flower extract concentration of 75% and 50%

with MRSA growth inhibition zone respectively 24.8 mm and 22.8 mm can be categorized strong inhibitory response. Meanwhile *rosella* flower extract concentration of 25% with MRSA growth inhibition zone 19.2 mm can be categorized medium inhibitory response whereas concentration of 12.5% MRSA growth inhibition zone 14.2 mm categorized weak inhibitory response.

Ability to inhibit the growth of MRSA by *rosella* flower extracts can not be separated from the content of its active compounds. Alkaloid compounds, flavonoids, tannins, saponins, and triterpenoids have antibacterial mechanism by damaging the cell wall structure and change the permeability of the cell cytoplasmic membrane (Dzulkarnain, Sundari and Chozin, 1996; Safera, 2005; Robinson, 1995). Changes and damage to the cytoplasmic membrane will cause leakage of intracellular materials and disruption of cell metabolism (Mukhlisoh, 2010).

Conclusions and Recommendations

Rosella flower extract (Hibiscus sabdariffa L.) has antibacterial activity against MRSA. Concentration of 25% is the concentration of the most effective in inhibiting the growth of MRSA as the smallest concentration that could produce inhibition zone diameter equivalent to 30 mg of vancomycin. The higher concentration of *rosella* flower extracts are given, the greater the inhibition zone diameter were formed.

Rosella flowers can be recommended for further investigation as antibacterial agents. Isolation of active compounds contained in the *rosella* flower needs to be done to determine the most effective compounds as anti-bacteria. Needs to be done to determine the dose of *rosella* flower extracts in vivo. There needs to be development of herbal medicine from *rosella* flower extract as an anti infection against bacterial that are resistant to current antibiotic selection.

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