



Phytochemical screening of bunga rosella (*Hibiscus sabdariffa* L) and antimicrobial activity test

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Abstract

The purpose of this study was to determine the antimicrobial activity of rosella infusa (*Hibiscus sabdariffa* L.). Antimicrobial inhibition test using infusa of rosella (*Hibiscus sabdariffa* L.) against *Staphylococcus aureus* and *Escherichia coli* bacteria at a concentration of infusa 100%; 75%; and 50%. The concentration has highest potential antibacterial of infusa for *Staphylococcus aureus* is 100% witch has diameter of 13.6 mm and for *Escherichia coli* bacteria is 100%, its show that diameter of zone inhibitory zone is 13.3 mm. The Results of phytochemical screening show that infusa of rosella (*Hibiscus sabdariffa* L.) contains flavonoids, saponins, and tannins. Based on the data above show that the infusa of rosella flower has high antibacterial potential.

Keywords: *Hibiscus sabdariffa* L., antimicrobial activity, *Staphylococcus aureus*, *Escherichia coli*

1. Introduction

Antibacterial activity from natural product has been growing rapidly the past few years. Bioactive compound of these natural product as antimicrobial activity has been publicated ([Gurning, 2020](#)). Rosella (*Hibiscus sabdariffa* L.) is a plant of the tropics and sub-tropics, including Indonesia. Rosella (*Hibiscus sabdariffa* L.) is one of those natural product which has high potential for herbal medicine. The application of rosella extract is as biosensor, antioxidant, indicator of acid-base, uric acid diet ([Tekerek et al. 2011](#); [Rahmah, 2017](#); [Amperawati et al. 2019](#); [Paristiowati et al. 2019](#)).

Staphylococcus aureus is a bacterial human pathogen. It causes a wide variety of clinical manifestations such as infection. Infections are common both in community-

acquired as well as hospital-acquired settings and treatment remains challenging to manage due to the emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant *Staphylococcus aureus*). These bacteria is found in the environment and normal human flora, located on the skin and mucous membranes (the nasal area) of most healthy individuals. *S. aureus* does ubnormally cause infection on healthy skin; however, if it is allowed to enter the bloodstream or internal tissues, these bacteria will cause a variety of potentially serious infections. Transmission is typically from direct contact. However, some infections involve other transmission methods (Franklin and Lowy, 1998; Boucher and Corey, 2008; Rasigade and Vandenesch, 2014; Jenul and Horswill, 2018; Paristiowati et al. 2019).

Staphylococcus aureus is a facultative anaerobic Gram-positif coccus, it is non motile and catalase and coagulase positive. It is cells are spherical single or paired cocci, or form grape-like clusters. Staphylococcal cell wall is resistant to lysozyme and sensitive to lysostaphin. *Staphylococcus aureus* strains are able to produce staphylococcal enterotoxins (SEs) and are causative agents of staphylococcal food poisonings. *Staphylococcus aureus* is found in the nostrils, and on the skin and hair of warm-blooded animals. Up to 30-50% of human population are carriers. *Staphylococcus aureus* is able to grow in a wide range temperatures 7°C- 48.5°C. Where the optimum growing is 30 to 37°C. On the acid condition is 4.2 to 9.3 pH with an optimum pH is 7 to 7.5. It still grow on the salt solution (sodium chloride) at 15% concentration. *Staphylococcus aureus* is an important pathogen due to combination of toxin-mediated virulence, invasiveness, and antibiotic resistance. This bacterium is a significant cause of nosocomial infections, as well as community-acquired diseases. The spectrum of staphylococcal infection Rsnge from pimples and furuncles to toxic shock syndrome and sepsis (Schmitt et al. 1990; Franklin and Lowy, 1998; Dinges et al. 2000).

Escherichia coli is one of the coliform group bacteria which has a capability live on the colon and human feses. It is gram negative bacteria that have a shape of a bull and do not form spores. *Escherichia coli* grows optimally at 38°C temperatures and can live in soil and water for months. This bacterium can die at a temperature of 60 for 20 minutes. Negative effect of these bacteria is caused infection such as pneumonia, urinary infection and wound infection (Dhafin, 2017; Febrina et al. 2017).

Rosella flower infusion can inhibit the growth of MRSA (Metichilin Resistant *Staphylococcus aureus*) clinical isolate collection of the Biomedical Research Unit of RSUP NTB (Pristianingrum et al. 2012). The average area of inhibition zone at a concentration of 100% is 8.7; 16; 18.3 mm for volume 50; 75; 100 µL and can be categorized in intermediates for volume 50 µL and sensitive for volumes 75 and 100 µL. According to Arifianti (2015) decoction of rosella flowers effectively became tooth decontamination material in testing the Minimum Inhibitory Concentration on the growth of *Escherichia coli* bacteria at a concentration of 0.5% and in the

Staphylococcus aureus bacteria at a concentration of 0.4%. On These research focus on the effect of Rosella Flower infusa for *Staphylococcus aureus* and *Escherichia coli* bacteria.

2. Methods

2.1 Preparation Sample

Fresh leaves of sample were taken and collected from Namorambe villages (district of Deli serdang) without regardless of the age and size of the leaves. The samples are cleaned by washing in running water, drained and dried in an open room that avoids direct contact with sunlight. The sample is pulverized using a blender.

2.2 Preparation of Infusa of Rosella

10 g of simplicia powder was mixture with aquadest up to 100 mL in a pan. Preheat and measure the temperature to 90°C occasionally stirred, after a temperature of 90°C let stand for 15 minutes, then served while still hot through flannel cloth, so Infusion results are obtained.

2.3 Phytochemical Screening of Simplisia and Infusa

Phytochemical screening from secondary metabolites of Rosella simplisia and infusa using standard phytochemical screening methods:

Alkaloids: The simplisia of Rosella 0,5 gr is dissolved in 1 mL HCl 2N and aquadest. The mixture is heated over a water bath for 2 minutes, cooled and filtered. The filtrate is divided into 3 equal parts. The first part drops some Mayers reagents; the second part with the Dragondroffs reagent and the third part with the Wagners reagent. Pay attention to sediments that are formed if orange deposits and brown deposits, indicate the presence of each alkaloids ([Vaghasiya et al. 2011](#)).

Tannins: The filtrate of the simplisia was obtained with a same procedure as above. 2 mL of the filtrate added with a few drops of alcoholic FeCl₃ reagent. The blue color indicates the presence of tannins ([Sitorus, 2018](#)).

Terpenoids and Steroids: As much as 1 gram of simplicia powder macerated with 20 ml of n-hexane for 2 hours, then filtered then the filtrate was evaporated and the remaining 20 drops of Lieberman-Burchard reagent were added, so that red or violet formed, this result showed a positive test for terpenoids, the formation of green or green blue shows positive test results for steroids ([Faskalia, 2014](#))

Flavonoids: A simplicia sample of 10 grams was added to 100 ml of hot water, then simmer for 5 minutes and filtered under heat, the filtrate obtained was taken 5 ml then added 0.1 gram of magnesium powder, 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, then shaken and allowed to separate. So that a red solution is formed indicating flavonoids ([Simaremare, 2014](#))

Saponin: A simplisia sample of 0.5 grams was put into a test tube, added with 10 ml of hot water and then cooled, shaken vigorously for 10 seconds to form a stable foam as high as 1-10 cm for not less than 10 minutes, and not lost with the addition of 1 drop of hydrochloric acid 2N indicates the presence of saponins ([Depkes RI, 1995](#)).

2.4 Characterization of Simplisia

Characterized of simplisia divided into 6 parameter: Mikroskopis test, Organoleptik test, Determination of total Ash, Determination of unsoluble acid total Ash, Determination of ethanol Soluble Sari Content Determination of Water Soluble Sari Content.

2.5 Preparation of Bacteria Suspended

The two solutions Sulfuric acid 1% 9,95 ml and $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ 1% 0,05 ml are mixed into a test tube and homogenized ([Dalynn, 2014](#)). Bacterial suspension is made by taking 4-10 ose of bacteria that have been rejuvenated for 24 hours into a test tube containing 0.9% NaCl, then homogenized. The suspension was incubated at 37°C for 24 hours. If the turbidity of the bacterial suspension solution is the same as the turbidity of the standard solution, the concentration of the bacterial suspension is 1.5×10^8 CFU / mL ([Zen et al. 2015](#)). The standardization of number of bacteria based on Mc. Farland suspend standart.

2.6 Minimum Inhibitory Concentration

Sterile NA media were put into sterile petri dishes as much as 20 mL each and allowed to condense at room temperature. The media was dropped with 1 mL of bacterial suspension test and flattened using an L rod until smooth and dry. Sterile disk paper with a diameter of 6 mm was dripped with 10 μL of rosella flower crown with 50%, 75%, 100% dosage variations, then placed on a solid agar media that had been dripped with test bacterial suspension, distilled water as negative control, and amoxicillin as positive control. Then incubated at 37°C for 24 hours and after incubation a clear zone was measured using calipers ([Ramadhani, 2015](#)).

3. Results and Discussion

3.1 Preliminary Phytochemical Screening

Phytochemical screening for metabolites secondary from Rosella simplisia and infusa flower is shown in [Table 1](#). Phytochemical screening results of simplisia and infusa showed contains has variety of secondary metabolites that are diverse.

3.2 Characterization of Simplisia

Characterization of simplisia using standar methods ([Febriani, 2015](#)). The result of characterization analysis of Rosella simplisia showed on bellow [Table 2](#).

Tabel 1

Phytochemical screening for metabolites secondary of Rosella (*Hibiscus sabdariffa* L.) simplisia and infusa

| Secondary Metabolites | Results | |
|----------------------------|-----------|--------|
| | Simplisia | Infusa |
| Alkaloids | + | - |
| Flavonoids | + | + |
| Saponin | + | + |
| Steroids and Triterpenoids | - | - |
| Tanin | + | + |

Table 2

Caracterization of Rosella (*Hibiscus sabdariffa* L.) flower Simplisia

| Parameters | Results |
|-----------------------------|---------------------|
| Oranoleptic | |
| Shape | Rough powder |
| Colours | Red to black |
| Odours | Smelled Distinctive |
| Taste | Acid |
| Mikroskopis | Trikoma, Kolenkim |
| Total Ash | 2.1% |
| Total Ash insoluble in acid | 0.5% |
| Soluble sari in ethanol | 12% |
| Soluble sari in water | 8.3% |
| %water | 6.7% |

3.3 Minimum Inhibitory Concentration

Antibacterial inhibitory activity test of rosella flower crown (*Hibiscus sabdariffa* L.) infusion was carried out by the 6 mm disk diffusion method against *Staphylococcus aureus* and *Escherichia coli* bacteria. The results of the antibacterial inhibitory activity of rosella flower crown (*Hibiscus sabdariffa* L.) against *Escherichia coli* and *Staphylococcus aureus* bacteria can be seen in the below [Table 3](#) and [Table 4](#).

Based on [Table 3](#) and [Table 4](#), it can be seen the criteria for antibacterial inhibition of roselle flower infusion (*Hibiscus sabdariffa* L.) against *Escherichia coli* bacteria and *Staphylococcus aureus* bacteria at 100% concentration and 75% concentration having strong antibacterial inhibition with inhibition zone diameter 13.3 mm, 10.5 mm in *Escherichia coli* bacteria and 13.6 mm, 10 mm in *Staphylococcus aureus* bacteria. While the 50% concentration has moderate inhibition criteria with a diameter of 8.1 mm in *Escherichia coli* bacteria and 8.8 mm in *Staphylococcus aureus* bacteria. Positive control namely Amoxicillin has a strong antibacterial inhibition with a diameter of

18.5 mm in *Escherichia coli* bacteria, whereas in *Staphylococcus aureus* bacteria has a very strong antibacterial inhibition with a diameter of 20.3 mm.

Table 3

Antibacterial activity test of rosella (*Hibiscus sabdariffa* L.) flower infusion against *Escherichia coli* bacteria

| Sample Variation | Inhibitory Diameter Zone (mm) | | | | Results |
|------------------|-------------------------------|------|------|---------|---------|
| | R1 | R2 | R3 | Average | |
| 50% | 7 | 8.5 | 9 | 8.1 | Middle |
| 75% | 10 | 10.5 | 11 | 10.5 | Strong |
| 100% | 12 | 13 | 15 | 13.3 | Strong |
| Positive control | 18 | 19 | 18.5 | 18.5 | Strong |
| Negative control | 0 | 0 | 0 | 0 | - |

R1 = Repeat 1; R2 = repeat 2; R3 = repeat 3

The antibiotic used is Amoxicillin as a positive control on *Escherichia coli* and *Staphylococcus aureus* bacteria. Amoxicillin is a broad-spectrum antibiotic, used for treatments such as infections of the airways, bile ducts, and arteries, gonorrhea, gastroenteritis, meningitis, and infections due to *Salmonella* sp, such as typhoid fever. The mechanism of action of amoxicillin is to inhibit bacterial cell wall synthesis by binding one or more of the penicillin-protein bonds so that it causes inhibition of cell wall biosynthesis so that bacteria break (Ariani et al. 2019; Siddiq, 2019). The active rosella (*Hibiscus sabdariffa* L.) crown infusion is said to be antibacterial due to the chemical components contained in the rosella flower crown (*Hibiscus sabdariffa* L.) plant. Based on the results of phytochemical screening that has been carried out the crown of roselle (*Hibiscus sabdariffa* L.) contains compounds flavonoids, saponins, and tannins.

Table 4

Antibacterial activity test of rosella (*Hibiscus sabdariffa* L.) flower infusion against *Staphylococcus aureus* Bacteria

| Sample Variation | Inhibitory Diameter Zone (mm) | | | | Results |
|------------------|-------------------------------|----|----|---------|-------------|
| | R1 | R2 | R3 | Average | |
| 50% | 8.5 | 9 | 9 | 8.8 | Middle |
| 75% | 10 | 9 | 11 | 10 | Strong |
| 100% | 12 | 14 | 15 | 13.6 | Strong |
| Kontrol Positif | 20 | 21 | 20 | 20.3 | Very strong |
| Kontrol Negatif | 0 | 0 | 0 | 0 | - |

Flavonoids have antibacterial activity because of the ability of flavonoids to interact with cell membranes and affect cell membrane bioactivity and it has been reported that flavonoids are able to reduce the fluidity of bacterial cell membranes that is directly related to damage to cytoplasmic membranes or indirect damage

through autolysis/weakening of the cell wall and consequently osmotic lysis (Gurning, 2020; Simanjuntak, 2020; Wu et al. 2013).

The mechanism of action of saponins as an antibakteri is by causing leakage of proteins and enzymes from the bacterial cell. Saponins are active substances that can increase membrane permeability resulting in the intercellular compound will diffuse through the outer membrane and cell wall (Gurning et al. 2019).

The mechanism of action of tannin as an antibacterial is by causing bacterial cells to become lysis. This happens because tannin has a target on the bacterial cell wall polypeptide wall so that the formation of the cell wall becomes less than perfect and then the bacterial cell will die. Tannins also have the ability to activate bacterial enzymes and interfere with the course of proteins in the inner layers of cells (Halimah et al. 2019).

4. Conclusion

Base on data show that antibacterial activity test of Infusa of Rosella (*Hibiscus sabdariffa* L.) has high potential antibacterial activity. It's showed the inhibitory diameter zone average of Infusa of Rosella against *Escherichia coli* and *Staphylococcus aureus* bacteria 13.3 mm and 13.6 mm respectively for 100% concentration of infusa of rosella.

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