Antibacterial effect potention of n-hexane fraction of rose apple leaves

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Abstract. This research was an attempt to prove scientifically about the antibacterial effect potention of n-hexane fraction of rose apple leaves [Eugenia aqueum (Burm. F) Alston]. The aims of this research were to determine the value of minimum inhibitory concentration (MIC) and type of effect as antibacterial from n-hexane fraction of rose apple leaves. Evaluation of antibacterial effect potention was carried out by diffusion method using well techniques on Staphylococcus aureus and Escherichia coli. Antibacterial effect was determined based on Bell's criteria. The type of work against bacteria was evaluated by turbidimetry method. The results showed that the n-hexane fraction of rose apple leaves have antibacterial effect both against S. aureus and E. coli. The MIC of n-hexane fraction of rose apple leaves on S. aureus and E. coli were 1.56% and 6.25% respectively. The type of effect of n-hexane fraction of rose apple leaves were bactericidal both against S. aureus and E. coli.

1. Introduction
Infectious diseases are still a health problem with a high burden based on the level of incidence and death in Indonesia [1]. Although there are many advances in the discovery of medicines for infectious diseases, in Indonesia, infectious diseases are still a major health problem. Some infectious diseases that were originally thought to have been controlled, now are re-emerging. This is caused by resistance from the infector to anti-infectives which have been commonly used, either because of patient misuse or due to mutations from the infector [2-4]. Efforts to explore new sources of natural agents that potential to be developed into drugs for infectious diseases, including those caused by bacteria, are very important [5].

Apple rose leaves have been known that have antibacterials effect. Ethanol extract of apple rose leaves had a great potential as an antimicrobial agent, although the MIC value of the apple rose leaves extract could not be determined. Research on the antibacterial activity of apple rose leaves carried out previously in 2015, only evaluates up to the level of extracts [6]. In connection with that, it is necessary to continue to the extract fraction level. Based on the results of the extract fraction level, any component in the apple rose leaves can be directed as an antibacterial component whether the components are polar, semi polar or nonpolar [7]. In this study, the antibacterial potential of the non-polar fraction, n-hexane, was evaluated.

Antibacterial type of effect is classified into bactericidal and bacteriostatic. The bactericidal type antibacterial is antibacterial which works by killing bacteria. Antibacterial or antibiotic type bacteriostatic work is antibacterial which works by inhibiting bacterial growth [8]. In this study the problems were determined as follows: does the n-hexane fraction of apple rose leaves had antibacterial
potential, what was the MIC value of the n-hexane fraction of apple rose leaves to the test bacteria and the type of effect.

2. Methods
The research phase included apple rose leaves collecting, simplicia, ethanol extract and n-hexane fraction making and finally, antibacterial activity test. The study was conducted at the Integrated Pharmacy Laboratory Unit D, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Bandung Islamic University. Ethanol extract was made by maceration using 96% ethanol [9]. The fraction was made using liquid-liquid extraction method using n-hexane [7]. Evaluation of antibacterial activity was carried out by the Kirby-Bauer method (disk diffusion method or agar diffusion) using hole (well) technique. Antibacterial activity was expressed by MIC [10]. Tests were carried out on E. coli that represented Gram negative bacteria [11,12] and S. aureus that represented Gram positive bacteria [2,13]. The results of this MIC determination were used to determine the sensitivity level of the test bacteria to the test agent based on the Bell criteria as stated in Table 1 [14]. An agent is said to have high antimicrobial potential if the MIC occurs at low concentration but has a large capacity to kill or inhibit growth test bacteria [6,8]. The sensitivity of a bacterium to a test agent can be determined based on Bell's criteria [14].

| Table 1. Categories of Sensitivity of Test Bacteria to an Agent Based on Bell’s Criteria [14]. |
|----------------------------------------------------------|
| Diameter (Ø) of the inhibitory zone | Sensitivity category |
| Ø ≥ 6 mm | Sensitive |
| Ø < 6 mm | Resist |

Based on the results of activity tests on both types of bacteria, the type of effect was determined. The type of effect against test bacteria was evaluated by turbidimetry method [15]. From this test will be obtained data on the bacterial growth based on time. By comparing bacterial growth curves that influenced by the test agent with normal bacterial growth curves it could be determined whether an agent have a type of bactericidal or bacteriostatic effect.

3. Results and discussion

3.1. Antibacterial test and determination of the MIC value
Based on the results of the observation, the diameter of inhibition zone data obtained from the n-hexane fraction of S. aureus and E. coli are listed in Table 2. The sensitivity of the test bacteria to the n-hexane fraction was determined based on the Bell criteria (Table 1). Based on Bell's criteria, the n-hexane fraction has anti-bacterial effect against S. aureus and E. coli. The MIC of ethanol extract of apple rose leaves against S. aureus was 3.13% while the MIC against E. coli was 1.56%. Because of the MIC against E. coli was lower than S. aureus, the n-hexane fraction had a stronger activity against E. coli than to S. aureus.

| Table 2. Diameter of the Inhibitory Zone of the n-Hexane Fraction against S. aureus and E. coli. |
|----------------------------------------------------------|
| Concentration (%) | S. aureus (cm) | E. coli (cm) |
|-------------------|---------------|-------------|
| 50                | 0.98 ± 0.07   | 1.01 ± 0.02 |
| 25                | 0.91 ± 0.01   | 1.04 ± 0.05 |
| 12.50             | 0.91 ± 0.03   | 0.99 ± 0.01 |
| 6.25              | 0.96 ± 0.06   | 1.04 ± 0.04 |
| 3.13              | 1.02 ± 0.01   | 1.00 ± 0.06 |
| 1.56              | -             | 0.92 ± 0.05 |
| 0.78              | -             | -           |
| 0.31              | -             | -           |
| 0.008% tetracycline | 1.76 ± 0.04 | 1.56 ± 0.78 |
3.2. Determination of type of effect

The type of effect of the n-hexane fraction in *S. aureus* and *E.coli* was determined at the MIC value obtained based on Bell's criteria (3.13% and 1.56% respectively). Based on Figure 1, it can be observed the growth of *S. aureus* growth up to 270 minutes of observation. The normal bacterial growth curve occurred up to the logarithmic phase. In the bacterial growth curve under the influence of the n-hexane fraction, there was no increase that showed no growth, even a decrease in 90 to 210 minutes. This showed a downward trend in growth. Thus the tendency of the working type of the n-hexane fraction to *S. aureus* was bactericidal, because after giving the test agent to the bacteria, the bacteria showed a tendency to decrease growth immediately.

![Figure 1. Development of *S. aureus* growth under the influence of n-hexane fraction based on observation of absorbance value.](image)

Based on Figure 2, it can be observed the development of *E. coli* growth up to 270 minutes of observation. The normal bacterial growth curve occurs up to the logarithmic phase. In *E. coli* growth curves which are under the influence of n-hexane fraction, there was an increase in absorbance from 30 to 60 minutes which resembles the logarithmic phase of the normal growth curve, but did not reach the stationary phase but immediately decreases until 180 minutes. The tendency of the working type of the n-hexane fraction of *E. coli* was bactericidal, because under the influenced by the fraction, after an absorbance increased until 60 minutes, the bacteria immediately showed a tendency to decrease growth. Figure 1 and 2 show that the n-hexane fraction curve was higher than the control. This happens because the color of the extract fraction was measured too.

![Figure 2. Development of *S. aureus* growth under the influence of n-hexane fraction based on observation of absorbance value.](image)
4. Conclusion
Based on the results of the study it can be concluded the antibacterial activity, minimum inhibitory concentration (MIC) and the type of work of the hexane fraction of guava leaves to S. aureus and E. coli. The n-hexane fraction of apple rose leaves has anti-bacterial activity against S. aureus and E. coli with a MIC value of 3.13% and 1.56%, respectively. The working type of the n-hexane fraction tends to be bactericidal both against S. aureus and E. coli.

Acknowledgments
Acknowledgments are conveyed to the Research and Community Service Institute of Bandung Islamic University for providing research funding.

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