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Activity of Nangka Kuning Extract (*Vincetoxcium villosum* (Blume) Kuntze) Against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract

Infectious diseases remain a major problem in many countries. The leaves of Nangka Kuning (*Vincetoxicum villosum* (Blume) Kuntze contain metabolites that have antibacterial properties. This research aims to determine the activity of chloroform extract of Nangka Kuning leaves (*Vincetoxicum villosum* (Blume) Kuntze) against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The extraction process in this study was carried out by maceration method using a chloroform solvent. The extraction results are then tested MIC with extract variations 12.5% - 25%. Then continue with the efficiency test. Second essay This do with to use method diffusion paper disk. The MIC test results showed that the chloroform extract of the leaves Nangka Kuning has antibacterial capacity against the bacteria *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 . Based on statistical analysis *Anova One Way* influence extract chloroform leaf nang ka dark yellow hinder bacteria *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 have brand Account F > Table F with value $\alpha = 0.05$. Follow-up of post hoc tests therefore to show that extract chloroform 15 % is the most effective concentration to inhibit the growth of *Staphylococcus aureus* ATCC 25923. And extract chloroform 17.5 % is the most effective concentration to inhibit the growth of *Pseudomonas aeruginosa* ATCC 27853.

Keywords: Nangka Kuning (*Vincetoxicum villosum* (Blumes)Kuntze) leaves, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

Full-length article **Corresponding Author*, e-mail: oktoviani@unib.ac.id

1. Introduction

Infectious diseases are still a major problem occurring in various countries, both in developing and developed countries, the World Health Organization (WHO) states that this disease is one of the causes of death among children. An infectious disease is an illness caused by the presence of microorganisms in the body such as bacteria, parasites, fungi, and viruses [1]. *Staphylococcus aureus* is a Gram-positive bacteria, hulls and bunches like grapes. *Staphylococcus aureus* is part of the normal flora present on the skin, but this bacterium can also be pathogenic. This bacterium can usually cause nosocomial infections originating from the upper respiratory tract, nose and throat, which can be caused by a "port of entry" from humans themselves [2]. *Pseudomonas aeruginosa* is a Gram-negative, aerobic, sporeless bacterium. This bacterium is an opportunistic pathogen that can cause invasive conditions in critically ill patients, this bacterium is also one of the bacteria responsible for nosocomial infection [3]. Nosocomial infection is one of the infection problems still quite widespread in the world. The incidence of this infection is quite high, namely 5% per year or 9 million out of 190 million patients treated. The mortality rate due to this infection reaches 1 million per year [4]. The management of infectious diseases caused by bacteria generally uses antibiotics. Widespread use of antibiotics can lead to antibiotic resistance. *The Central for Disease Control and Prevention* (CDC) states that approximately 2 million people are infected with bacteria and have antibiotic resistance, and 23,000 people have died due to antibiotic resistance [5].

This problem can be overcome by using herbal ingredients found in nature to overcome or reduce the occurrence of antibiotic resistance [6]. There are many advantages to using plant-based ingredients, including that they are easy to obtain, safer, and do not cause resistance. In the present study, one of the candidate plants to be used is Nangka Kuning leaves (*Vincetoxicum villosum* (Blume) Kuntze) [7]. Nonpharmacological treatment have lower level of danger, side effects and risk than synthetic chemical drugs [8]. Phytochemical test results from previous research showed that the chloroform extract of Nangka Kuning leaves contained secondary metabolite compounds in the form of alkaloids [9]. Previous research comparing the activity of chloroform extract and ethanol extract from seeds *Strychnos*

ligustrin BI showed that chloroform extract had better activity against bacteria *Staphylococcus aureus* and *Salmonella thypi* [6]. Based on this background, the author then had the idea to research the activity of chloroform extract of Nangka Kuning leaves (*Vincetoxicum villosum* (Blume) Kuntze) against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

2. Materials and Methods

The materials used are Nangka Kuning leaves, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, chloroform solvent, chloramphenicol antibiotic, sterile distilled water, alcohol, trypticase soy broth and trypticase soy agar. The Nangka Kuning leaves used in this research went through several stages. The first stage is the drying process, this process is carried out using the wind-air method. This procedure aims to reduce the water content in yellow jackfruit leaves. During the drying process, it is important to always pay attention to temperature, technique and time, with the aim of ensuring that the active compound content in the leaves is not damaged [10]. The drying process is carried out by avoiding direct exposure to sunlight to ensure that the active compound content in the leaves is not damaged by UV rays. This research uses an experimental analytical study type research conducted in a laboratory. This antibacterial activity test used various concentrations of Nangka Kuning (*Vincetoxicum villosum* (Blume) Kuntze) leaf chloroform extract using disk paper and examined the zones of inhibition formed. The data used in this research is primary data. The data comes from research carried out directly in the laboratory by measuring the growth inhibition zone of *Staphylococcus aureus* and *Pseudomonas aeruginosa* on TSA media having received chloroform extract of Nangka Kuning leaves (*Vincetoxicum villosum* (Blume) Kuntze).

The extraction process in this research was carried out using the method of maceration using a chloroform solvent. The results of the extraction are then tested *Minimum Inhibitory Concentration* (MIC) with extract variations 12.5% - 25%. The determination of the MIC was carried out by examining the minimum concentration capable of inhibiting bacterial growth, seen by the size of the diameter of the inhibition zone and its growth which had been incubated for 24 hours [11]. Then continue with the efficiency test. These two tests were carried out using the paper disk diffusion method. The parameter used in this research is the inhibition zone formed around the paper disc. The comparison solution used for this research was the antibiotic chloramphenicol.

3. Results and Discussions

This research received ethical clearance from the Faculty of Medicine and Health Sciences, Bengkulu University ethics no. 98/UN30.14.9/LT/2022. 2 kg of Nangka Kuning leaves obtained in Lahat, South Sumatra with the criteria of yellow-green leaves and in a fresh state, will be air-dried to obtain 250 g of dry leaves. Then, a maceration will be carried out to obtain a filtrate of 0.5 liters. The filtrate obtained will be evaporated using *a rotary evaporator* and *bain-marie* until you get a thick paste. The yield value of the chloroform extract of Nangka Kuning leaves was 4.19%. The data obtained from the MIC test results will then be statistically analyzed. The normality of the data obtained will first be tested using the *Shapiro-Wilk test* with a result of $p > 0.05$ so that the data is normally distributed. Additionally, the data will be tested using *Levene's test* to test the homogeneity of the data, so that the results obtained are $p = 0.74$ ($p > 0.05$). The results of the ANOVA test analysis showed that the value of $p = 0.000$ ($p < 0.05$), which means that there is a significant difference in the average diameter of the inhibition zone for each concentration. The Tukey HSD *post hoc* test was used to analyze comparisons between treatment groups with significant differences. *Post hoc* which was carried out, it can be concluded that there are significant differences between the inhibition zones formed by the chloroform extract of Nangka Kuning leaf at concentrations of 12.5%, 15%, 17.5%, 20%, 22.5% and 25% in the control (+), which means that the average inhibition zone formed from all concentration variations is even smaller than the inhibition zone formed in the control (+) with an average zone of inhibition value of 10.83 mm. And there was a significant difference between the inhibition zone formed by chloroform extract of Nangka Kuning leaves at concentrations of 12.5%, 15%, 17.5%, 20%, 22.5% and 25%. % compared to the control (-) where the results were obtained if the average diameter of the inhibition zone formed by varying the concentration of the chloroform extract of Nangka Kuning leaves is greater than the average diameter of the inhibition zone control (-) which has an average value of 0.00 mm (Figure 1). The data obtained from the MIC test results will then be statistically analyzed. The normality of the data obtained will first be tested using the *Shapiro-Wilk test* with a result of $p > 0.05$ so that the data are normally distributed. Next, the data will be tested using *Levene's test* to test the homogeneity of the data, so that the result is $p = 0.102$ ($p > 0.05$). The results of the ANOVA test analysis showed that the value of $p = 0.000$ ($p < 0.05$), which means that there is a significant difference in the average diameter of the zone of inhibition for each concentration. The *post hoc Tukey HSD* test was used to analyze comparisons between treatment groups with significant differences.

Post hoc Tukey HSD test carried out concluded that there were significant differences between the zones of inhibition formed by the chloroform extract of Nangka Kuning leaves at concentrations of 12.5%, 15%, 17.5 %, 20%, 22.5%, and 25% compared to control (+) where the average size of the zone of inhibition formed from all concentration changes is even smaller than the zone of inhibition formed in control (+) with an average inhibition zone value of 10.00 mm. Significant differences also occurred between the zones of inhibition formed by the chloroform extract of Nangka Kuning leaf at the concentrations of 12.5%, 15%, 17.5%, 20%, 22.5% and 25%. compared to control (-) where results were obtained if the average diameter of the zone of inhibition formed by various concentrations of chloroform extract of Nangka Kuning leaves was greater than the average diameter of the control zone of inhibition (-), which had an average value of 0.00 mm (Figure 2). Based on the results of the MIC test of the chloroform extract of yellow nanga leaves against the bacterium *Staphylococcus aureus* ATCC 25923, it was found that the extract with concentrations of 12.5%, 15%, 17.5% , 20%, 22.5%, 25% , had moderate inhibitory potency, with the highest inhibitory potency at 15% concentration of 7.6 mm.

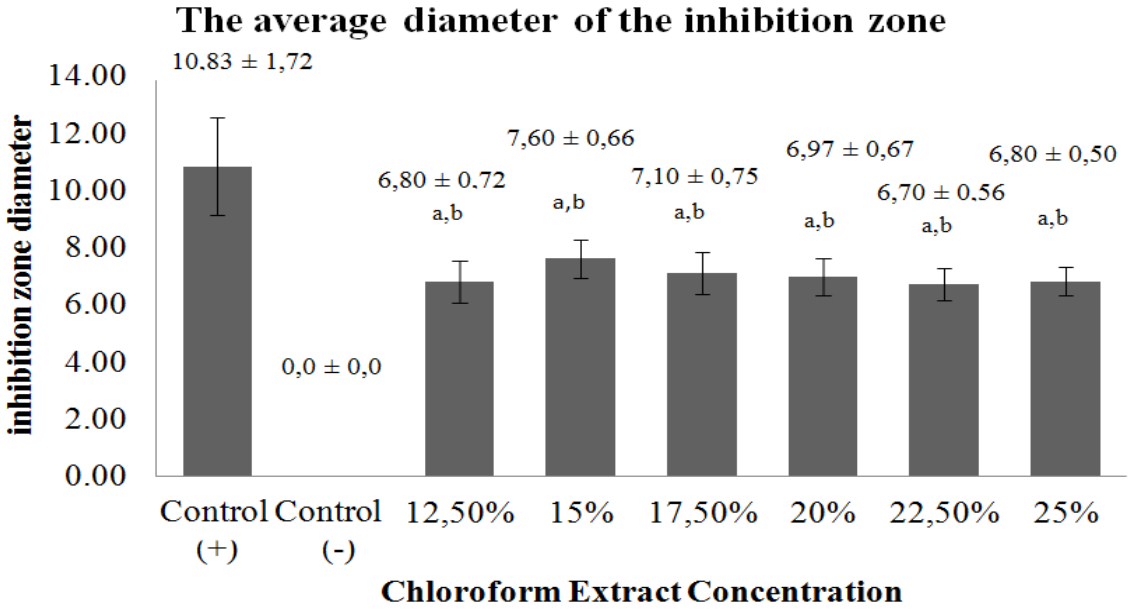

Figure 1: Average diameter of the zone of inhibition Chloroform extract of Nangka Kuning leaves against the bacterium *Staphylococcus aureus* ATCC 25923

Information: a: Significant difference from control (+), b: Significant difference compared to the control (-)

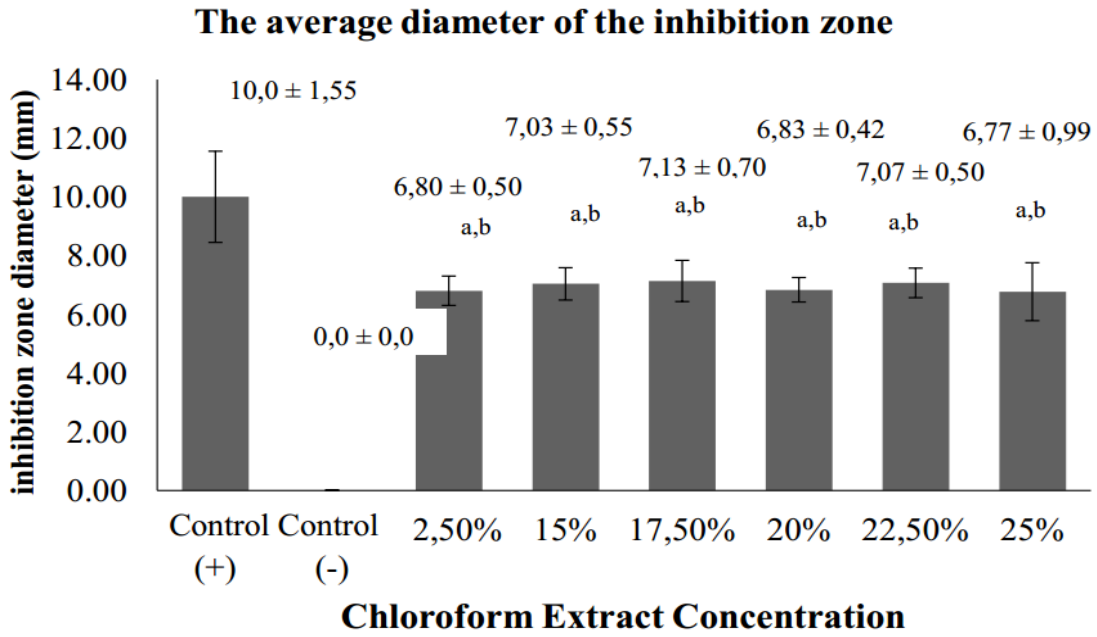

Figure 2. Average diameter of the zone of inhibition of the chloroform extract of Nangka Kuning leaves against the bacterium *Pseudomonas aeruginosa* ATCC 27853

Information: a: Significant difference from control (+), b: Significant difference compared to the control (-)

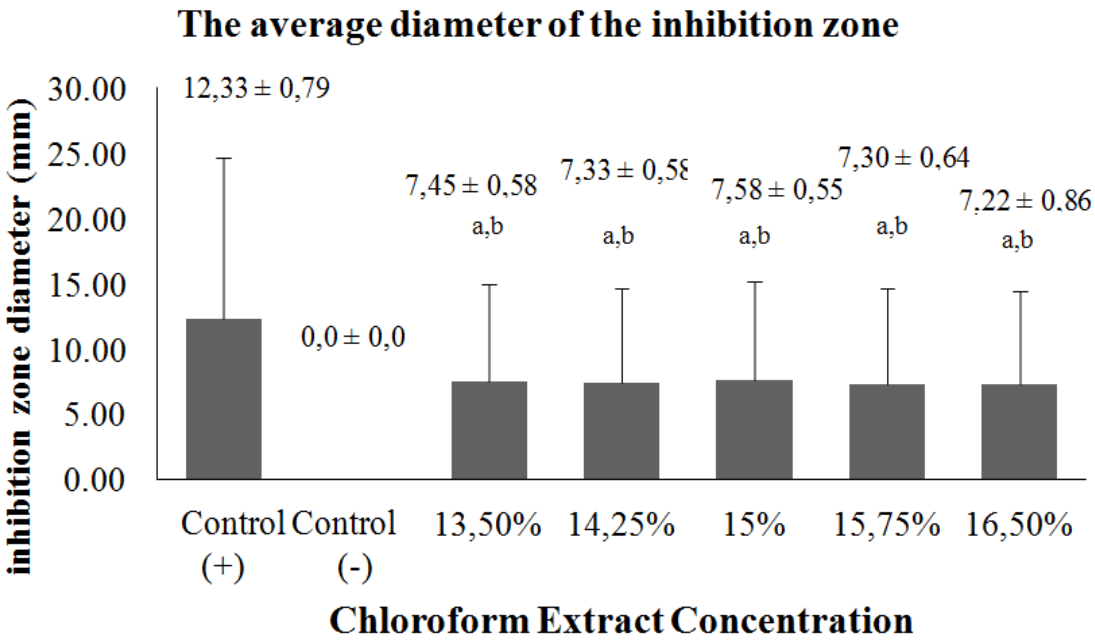

Figure 3. Average diameter of the zone of inhibition of the chloroform extract of Nangka Kuning leaves against the bacterium *Staphylococcus aureus* ATCC 25923

Information: a: Significant difference from control (+), b: Significant difference compared to control control (-)

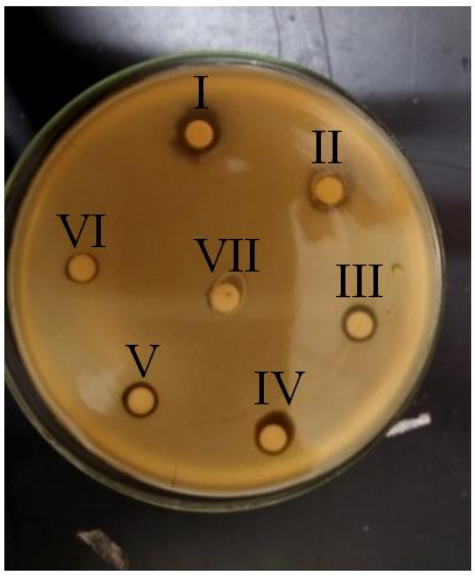

Figure 4. Average diameter of the inhibition zone of chloroform extract of Nangka Kuning leaves against the bacterium *Staphylococcus aureus* ATCC 25923

Information:

I: Control + (Chloramphenicol), Diameter of the zone of inhibition 12.33 mm (strong)
II: Concentration 13.5%, Diameter of the zone of inhibition: 7.45 mm (moderate)
III: Concentration 14.25%, Diameter of the inhibition zone: 7.33 mm (moderate)
IV: Concentration 15%, Diameter of the inhibition zone: 7.58 mm (moderate)
V: Concentration 15.75%, Diameter of the inhibition zone: 7.30 mm (moderate)
VI: Concentration 16.5%, Diameter of the inhibition zone: 7.22 mm (moderate)
VII: Control - (Vegetable Oil), Diameter of the inhibition zone: 0 mm (none)

The zone of inhibition formed against the bacterium *Pseudomonas aeruginosa* ATCC 27853 with concentrations of 12.5%, 15%, 17.5%, 20%, 22.5%, 25%, has a moderate classification of inhibitory power, with the highest inhibitory power at a concentration of 17. 0.5% of 7.1 mm. The next step after obtaining the best concentration of zone of inhibition formed in the MIC test of chloroform extract of Nangka Kuning leaves against Staphylococcus *aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853, then an efficacy test was carried out by varying the concentration of the extract which was higher and lower than the best concentration. (Appendixes 7 and 8), namely for the chloroform extract against *Staphylococcus aureus* ATCC 25923, i.e., a concentration of 15% (A3) with a distance of 0.75% so that a new concentration of 13.5%, 14.25%, 15%, 15 is obtained. 75% and 16.5%. Chloroform extract against the bacteria *Pseudomonas aeruginosa* ATCC 27853, namely a concentration of 17.5% (A3) with a distance of 0.75%, so that new concentrations of 16%, 16.75%, 17.5% , 18.25% and 19% were obtained. The positive control used was the antibiotic chloramphenicol. The next step after obtaining the best concentration is then classified into weak, medium, strong or very strong categories, based on the classification of David and Stout (1971). In this study, a concentration of 15% was obtained as the best concentration for *Staphylococcus aureus* ATCC 25923, then a dilution of 0.75% was carried out to obtain a concentration of 13.5% (A1), 14.25% (A2), 15% (A3), 15.75% (A4) and 16.5% (A5). Meanwhile, the concentration of the extract for the bacteria *Pseudomonas aeruginosa* ATCC 27853 obtained concentrations of 16% (A1), 16.75% (A2), 17.5% (A3), 18.25% (A4) and 19% (A5). Both used a chloramphenicol positive control and a vegetable oil negative control. The data obtained from the results of the effectiveness tests will then be analyzed statistically. The normality of the data obtained will first be tested using the *Shapiro-Wilk test* with a result of $p > 0.05$ so that the data are normally distributed. In addition, the data will be tested using *Levene's test* to test the homogeneity of the data, so that the results obtained are $p = 0.123$ ($p > 0.05$). Analysis of the Anova test showed that the value of $p = 0.000$ ($p < 0.05$), which means that there is a significant difference in the average diameter of the zone of inhibition for each concentration. The Tukey HSD *post hoc* test was used to analyze comparisons between treatment groups with significant differences.

Post hoc for chloroform extract of Nangka Kuning leaves against the bacterium *Staphylococcus aureus* ATCC 25923 can be concluded if there is a significant difference between the inhibition zone formed by the chloroform extract of Nangka Kuning leaves at the concentrations of 13.5%, 14.25%, 15%, 15.75% and 16.5% compared to the control. (+) where the average size of the inhibition zone formed from all concentration variations is even smaller than the inhibition zone formed in the control (+) with an average inhibition zone value of 12 .33mm. There was also a significant difference between the inhibition zone formed by the chloroform extract of Nangka Kuning leaves at the concentrations of 13.5%, 14.25%, 15%, 15.75% and 16.5%, compared to the control (-) where the results were obtained if the average diameter of the inhibition zone formed by varying the concentration of chloroform extract of Nangka Kuning leaves is greater than the average diameter of the control inhibition zone (-) which has an average value of 0.00 mm (Figure 3 and 4).

Tukey post hoc HSD chloroform extract of Nangka Kuning leaves against the bacterium *Pseudomonas aeruginosa* ATCC 27853 which was performed concluded that there was a significant difference between the zone of inhibition formed by the chloroform extract of Nangka Kuning leaves at concentrations of 16%, 16.75%, 17.5 %, 18.25% and 19% compared to the control (+) where the average size of the zone of inhibition formed from all the concentration changes is even smaller than the zone of inhibition formed in the control (+), the average value of the zone of inhibition formed being 12.20 mm. There were also significant differences between the zones of inhibition formed by the chloroform extract of Nangka Kuning leaf at concentrations of 16%, 16.75%, 17.5%, 18.25% and 19% compared to the control (-) where the results were obtained if the On average, the average diameter of the zone of inhibition formed by the variations in the concentration of chloroform extract of Nangka Kuning leaves was greater than the average diameter of the zone d control inhibition (-) with a average value of 0.00 mm. It was concluded that the average zone of inhibition formed by the chloroform extract of Nangka Kuning leaves against the bacteria *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 was included in the medium category, based on the classification of David and Stout (1971). The average zone of inhibition of Nangka Kuning leaf chloroform extract against Staphylococcus *aureus* ATCC 25923 with concentration of 13.5%, 14.25%, 15%, 15.75% and 16.5% was included in the medium category, and for the zone of inhibition, the medium chloroform extract of Nangka Kuning leaves against the bacterium *Pseudomonas aeruginosa* ATCC 27853 with concentrations of 16%, 16.75%, 17.5%, 18.25% and 19% included in moderate. It was used to determine the mean difference of chloroform extract against the bacterium *Staphylococcus aureus* ATCC 25923 in the parametric Anova test with concentrations consisting of Control (+), Control (-), 13.5%, 14.25 %, 15%, 15.75% and 16.5%, ss so that a calculated F value of 132.423 is obtained with an F table of 2.57 and an ap (Sig.) value of 0.000. The calculated F value result is greater than the F table or p value <0.05, so at a significance level of 5% (0.05), Ho is rejected, meaning there is a difference significant (significant). Results Efficacy against *Staphylococcus aureus* ATCC 25923 each concentration composed of Control (+), Control (-), 13.5%, 14.25%, 15%, 15.75% and 16.5%.

Chloroform extract against the bacterium *Pseudomonas aeruginosa* ATCC 27853 with the concentrations of Control (+), Control (-), 16%, 16.75%, 17.5%, 18.25% and 19% I got an F count value of 181.168 with an F array of 2.57 and a p-value (Sig.) of 0.000. The calculated F value result is greater than the F table or p value <0.05, so at a significance level of 5% (0.05), Ho is rejected, meaning there is a difference significant (significant). Results Efficacy against *Pseudomonas aeruginosa* ATCC 27853, each strength consisting of Control (+), Control (-), 16%, 16.75%, 17.5%, 18.25% and 19%. The inhibition zone formed in the effectiveness test of Nangka Kuning leaves against the bacteria *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 shows that the diameter of the inhibition zone formed is not always directly proportional to the increase in extract concentration. There are several factors that cause this, including differences in the speed of antibacterial compounds on the agar media as well

as different types and concentrations of antibacterial compounds, as well as the thickness of the agar media used [12]. Each secondary metabolite content in Nangka Kuning leaves has its own mechanism for inhibiting the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria. In this research, it is known that the chloroform extract of nangka Kuning leaves contains metabolite compounds in the form of alkaloids, where the mechanism of alkaloids in inhibiting growth is by interfering with the components that make up the peptidoglycan in bacterial cells so that the cell wall layer does not form completely and causes death of the bacterial cells [13].

Furthermore, after the effectiveness test results had been obtained, they were analyzed using SPSS 26.0 with the One Way Anova method, resulting in a calculated F that was greater than the F table, at the 0.05% level, which means the hypothesis could be accepted at the 95% level. Apart from that, in this test it was also explained that there were significant differences in the inhibition zone produced by various variations in the concentration of Nangka Kuning leaf chloroform extract. Chloroform extract of Nangka Kuning leaves produced greater inhibition on the bacteria *Staphylococcus aureus* ATCC 25923 than *Pseudomonas aeruginosa* ATCC27853. This shows that the chloroform extract of Nangka Kuning leaves is more effective against *Staphylococcus aureus* bacteria, which are gram-positive bacteria, compared to *Pseudomonas aeruginosa* bacteria, which are gram-negative bacteria. Gram-negative bacteria have a complex cell wall structure compared to gram-positive bacteria so that secondary metabolite compounds such as alkaloids found in extracts more easily penetrate and damage the cell walls of gram-positive bacteria [14].

4. Conclusions

Chloroform extract of Nangka Kuning leaves (*Vincetoxicum villosum* (Blumes) Kuntze) against the bacterium *Staphylococcus aureus ATCC 25923* had the best antibacterial activity at a concentration of 15% with a mean zone of inhibition value of 7.58 mm. Chloroform extract of Nangka Kuning leaves (*Vincetoxicum villosum* (Blumes) Kuntze) against the bacterium *Pseudomonas aeruginosa* ATCC 27853 shows the best antibacterial activity at a concentration of 16% with a mean zone of inhibition value of 6.85 mm. Chloroform extract of Nangka Kuning leaves (*Vincetoxicum villosum* (Blumes) Kuntze) has antibacterial activity against the bacteria *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. The zone of inhibition was included in the medium category and smaller than chloramphenicol as a positive control.

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