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**Antibacterial Activity Of *Staphylococcus aureus* From Chinese Betel Leaf Juice (*Peperomia pellucida* L.)**

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Accepted: 12 September 2024; Approved : 25 Oktober 2024

**ABSTRAK**

Penyakit infeksi, khususnya di negara berkembang seperti Indonesia, menjadi masalah kesehatan yang sering dihadapi, salah satunya disebabkan oleh bakteri. Penelitian ini menguji aktivitas antibakteri sari daun sirih cina terhadap bakteri *Staphylococcus aureus*, dengan kandungan metabolit sekunder seperti alkaloid, tanin, dan flavonoid yang berperan sebagai antibakteri. Penelitian ini merupakan eksperimen laboratorium menggunakan rancangan acak lengkap dengan tiga kali pengulangan pada lima konsentrasi sari, serta satu kontrol positif dan negatif. Metode yang digunakan adalah metode difusi agar, dan hasil pengujian menunjukkan bahwa sari daun sirih cina memiliki potensi antibakteri terhadap *Staphylococcus aureus* pada berbagai konsentrasi yang diuji. Konsentrasi yang digunakan adalah 6µL/disc, 8µL/disc, 10µL/disc, 12µL/disc, dan 14µL/disc, di mana semua kategori ini menghasilkan zona hambat yang termasuk dalam kategori sedang menurut Susanto (2012). Analisis statistik menggunakan uji Kruskal-Wallis menghasilkan nilai signifikan 0,035 (< 0,05), menunjukkan adanya perbedaan signifikan antara masing-masing konsentrasi yang diuji. Dari hasil penelitian, dapat disimpulkan bahwa sari daun sirih cina memiliki aktivitas antibakteri yang efektif terhadap *Staphylococcus aureus*.

**Kata kunci:** Antibakteri, *Staphylococcus aureus*, *Peperomia pellucida* L.

**ABSTRACT**

Infectious diseases, especially in developing countries such as Indonesia, are a common health problem, one of which is caused by bacteria. This study tested the antibacterial activity of Chinese betel leaf extract against *Staphylococcus aureus* bacteria, with secondary metabolite content such as alkaloids, tannins, and flavonoids that act as antibacterials. This study was a laboratory experiment using a completely randomized design with three repetitions at five concentrations of extract, as well as one positive and negative control. The method used was the agar diffusion method, and the test results showed that Chinese betel leaf extract had antibacterial potential against *Staphylococcus aureus* at various concentrations tested. The concentrations used were 6µL/disc, 8µL/disc, 10µL/disc, 12µL/disc, and 14µL/disc, where all these categories produced inhibition zones included in the moderate category according to Susanto (2012). Statistical analysis using the Kruskal-Wallis test produced a significant value of 0.035 (<0.05), indicating a significant difference between each concentration tested. From the research results, it can be concluded that Chinese betel leaf extract has effective antibacterial activity against *Staphylococcus aureus*.

**Keywords:** Antibacterial, *Staphylococcus aureus*, *Peperomia pellucida* L.

**1. INTRODUCTION**

Infectious diseases, especially in developing countries such as Indonesia, are one

of the health problems that many people face today. One of the factors causing this infection is the presence of bacteria<sup>1</sup>. Skin and soft tissue

infections occur in 10% of cases of bacterial infections being the cause of hospitalization. Most bacterial infections of the skin and soft tissues are resolved within 7 to 10 days, it is difficult to estimate the exact incidence of skin and soft tissue infections as the clinical manifestations and duration of infection vary<sup>2,3</sup>.

The incidence of the disease is increasing due to an aging population and people with serious illnesses. Other causes of increased incidence include increased use of immunosuppressive drugs, malignancies, organ transplants, medical interventions, and surgical wound infections<sup>2</sup>.

*Staphylococcus aureus* is known as a pathogenic bacterium that infects humans. In addition, this bacterium is an opportunistic pathogen and commensal bacterium<sup>4</sup>. These bacteria cause infections that spread to blood vessels and produce toxins resulting in shock syndrome<sup>5</sup>. This type of bacteria is gram-positive and occurs naturally in various parts of the human body, including the skin, mucous membranes of the nose, mouth and colon. When a person's immune system is weakened, the bacteria can become pathogenic and cause boils, abscesses, and various inflammation-related infections<sup>6</sup>.

Empirically in the Minahasa area, especially in Lemoh Village, Chinese betel leaf (*P. pellucida* L.) is used by the local community as a traditional prevention and treatment of infectious diseases caused by bacteria by taking the leaves of the Chinese betel leaf plant with a hand grip and then mashing it until smooth after that it is attached to the injured skin surface area. Therefore, it is necessary to prove on a laboratory scale whether Chinese betel leaves have antibacterial activity.

## 2. RESEARCH METHODS

### Place and Time of Research

This research was conducted at the Pharmaceutical Biology and Pharmaceutical Chemistry laboratories of the Faculty of Mathematics and Natural Sciences, Universitas Kristen Indonesia Tomohon. The research was conducted in January 2023-March 2024.

### Tools and Materials

The tools used in this study are: Laminar air flow, autoclave, desiccator, incubator, ultra violet lamp, scale, beaker glass, measuring cup, bunsen, erlenmayer, test tube, chamber, silica 60

GF254 KLT plate, capillary pipette, cotton, sterile gauze, aluminum foil, mortar and pestle, 10cm petri dish, gloves, mask, lab coat, stationery, camera, container, knife, 8mm paper disc, spatula, tweezers, pipette, 1.5ml eppendorf.

The materials used in this study are: Chinese betel leaf (*P. pellucida* L.), *Staphylococcus aureus* bacteria (ATCC 25923) obtained from the Laboratory of the Faculty of Mathematics and Natural Sciences, Christian University of Indonesia Tomohon, Nutrient Agar (NA), Nutrient Broth (NB), 95% alcohol, distilled water, ampicillin, McFarlan 0.5 standard solution, ethyl acetate, n-hexane, chloroform, dragendoff, FeCl<sub>3</sub> 5%, ammonia, methanol.

### Research Type and Research Design

This type of research is laboratory experimental research, using a Completely Randomized Design (CRD) by doing three repetitions on 5 concentration series, 1 positive control and 1 negative control for one type of bacteria, *Staphylococcus aureus*. The antibacterial test method used in this study is the diffusion method with the Kirby Bauer technique, namely the agar diffusion method with paper discs. The concentrations of Chinese betel leaf juice (*P. pellucida* L.) used were: 6µL/disc, 8µL/disc, 10µL/disc, 12µL/disc and 14µL/disc. The positive control used was 10µL/disc ampicillin antibiotic and the negative control was sterile distilled water.

### Research Procedure

#### Tool Sterilization

The tools to be used were sterilized using an autoclave for 30 minutes at 121°C with a pressure of 1 atm. Sterilization is an activity whose purpose is to kill microorganisms. In the process of making media, sterilization of tools is carried out to avoid contamination<sup>7</sup>.

#### Chinese Betel Leaf Sampling

The samples used in this study were fresh green Chinese betel leaves obtained from Lemoh Village, Minahasa Regency which were taken by hand. Samples of Chinese betel leaves that have been taken, collected, wet sorted / cleaned from impurities, washed under running water and drained to reduce water content, then mashed using a mortar and pestle, then placed in sterile gauze and then squeezed to get Chinese betel

leaf juice. Then the juice obtained is transferred into a 1.5ml Eppendorf.



**Figure 1.** Chinese Betel Leaf Plant

### Identification of Compounds by Thin Layer Chromatography

#### a. Alkaloids

Samples were photographed on a TLC plate with a mobile phase of n-hexane: ethyl acetate (7: 3) v/v with dragendoff stain reagent after being sprayed with dragendoff reagent, it produces orange or brownish red spots if it is positive for alkaloid compounds.

#### b. Flavonoids

Samples were photographed on a TLC plate with a mobile phase of methanol: chloroform (1:9) v/v with an ammonia vapor stain identifier. If it is positive for flavonoid compounds, it will be blue when viewed at UV lamp 366 nm and yellow after ammonia evaporation.

#### c. Tannins

Samples were photographed on a TLC plate with a mobile phase of n-hexane: ethyl acetate (6: 4) v/v and 5% FeCl<sub>3</sub> stain reagent. If it is positive for tannin compounds, then a black colored stain is formed.

$$R_f = \frac{\text{Distance traveled by stain}}{\text{Distance traveled by eluent}}$$

Then the R<sub>f</sub> value or retention factor of each spot that appears and is colored is calculated<sup>8</sup>.

### Preparation for Test Bacteria Suspension

The test bacteria were taken and then suspended into an Erlenmeyer containing 100 ml of distilled water with 0.8g of sterilized NB and then stirred, then the bacterial suspension was incubated at 30°C for 24 hours. Bacterial growth is characterized by turbidity in the media. After incubating for 24 hours, it was taken and shaken

until homogeneous. Then the level of turbidity is seen, namely comparing according to the McFarland 0.5 standard solution visually.

### Positive Control Preparation

The positive control solution to be used is ampicillin (500<sup>®</sup>), with a concentration of 10μL/disc. This solution was made by grinding ampicillin tablets using a mortar and pestle and then weighing them to obtain ampicillin powder equivalent to 50 mg and dissolving it in 50ml of distilled water.

### Solid Media Preparation

The solid medium used is Nutrient Agar (NA). This solid media is made by weighing 2.07 grams of Nutrient Agar (NA) media then dissolving it with 90 mL of distilled water in an Erlenmeyer, stirring until the powder dissolves. Next, it was sterilized using an autoclave at 121°C for 15 minutes.

### Antibacterial Test Procedure

Pipette 1 mL of bacterial suspension into an erlenmeyer containing 30 mL of NA that is already warm (temperature 44°C - 50°C). Then stirred until homogeneous and then poured into a petri dish that has been sterilized, under aseptic conditions, then left to solidify. The disc paper that has been saturated with the test solution has previously been allowed to dry at room temperature. Samples were prepared by pipetting 6μL, 8μL, 10μL, 12μL, and 14μL in the disc paper. The ampicillin antibiotic solution as a positive control was pipetted as much as 10μL, and as a negative control a paper disk that had been pipetted with 6μL of sterile distilled water was used. Then the dried disc paper is affixed to the test media in a Petri dish that has been marked then incubated at 34°C- 37°C for 24 hours in an inverted position. Furthermore, after 24 hours of incubation, observe whether there is an inhibition zone formed around the disc paper, measure the inhibition zone formed using a caliper and do this test 3 times.

### Data Analysis

After the data is collected, the results of observations and measurements are tabulated in tables and figures<sup>9</sup>. Testing the antibacterial activity of Chinese betel leaf juice against *staphylococcus aureus*. Subsequently, it was analyzed statistically. Using the Kruskal-Wallis non-parametric test and further analysis using

Mann Whitney analysis with the Statistical Product Service Solution (SPSS) program with a confidence level of 95% or  $\alpha = 0.05$ <sup>10</sup>.

Inhibition zone diameter calculation<sup>11</sup>.

$$\text{Formula: } D = \frac{A+B+C}{3} \dots\dots\dots (1)$$

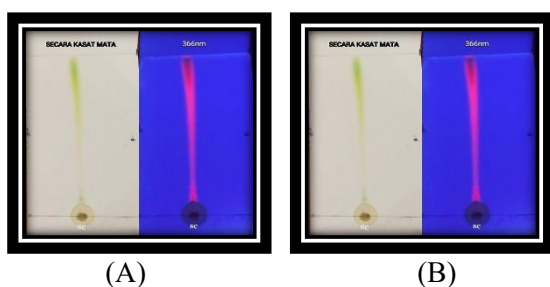
Description:

- D: diameter of inhibition zone
- A: vertical diameter
- B: horizontal diameter
- C: diameter diagonal

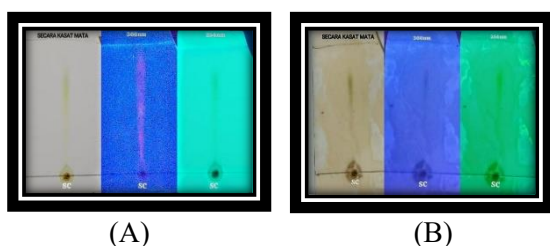
**3. RESULTS AND DISCUSSION**

**Thin Layer Chromatography (TLC)**

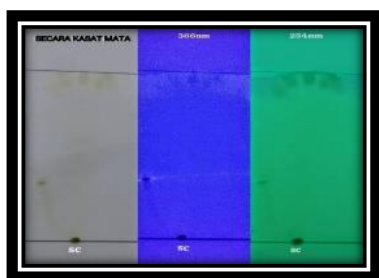
TLC result:



**Figure 2.** TLC Results of Alkaloids (A) Before being sprayed with Reagent, (B) After being sprayed with Dragendoff Reagent



**Figure 3.** TLC Results of Tannin (A) Before being sprayed with Reagent, (B) After being sprayed with 5% FeCl3 Reagent



**Figure 4.** Flavonoid TLC Results After Evaporation of Ammonia Reagent

The respective results obtained in this test are:

a. Alkaloids

Observations on the TLC plate before being given the reagent and UV irradiated produced yellow green stain spots, after UV 366 nm irradiation revealed orange, fluorescent stain spots. Furthermore, the results given by the dragendoff reagent obtained results before UV irradiation are green and at UV 366 nm the results are blackish orange and at UV 254 nm get the results of green stain spots. By using the mobile phase which is n-hexane: ethyl acetate (7:3). The appearance of stains on the KLT plate before and after giving dragendoff reagent with a brownish green or brownish orange color is positive for alkaloid compounds<sup>12</sup>.

The color that appears and is formed due to the coordinate covalent bonds in the K<sup>+</sup> ions of potassium tetraiodobismutate forming an alkaloid potassium complex<sup>13</sup>. The n-hexane eluent is a non-polar solvent while ethyl acetate is a polar solvent. The eluent can separate many stains and there are stains that indicate the presence of alkaloid compounds<sup>14</sup>. So, the results of this study indicate that Chinese betel leaf juice is detected to have tannin alkaloid compounds. The TLC results showed yellow green stain spots before UV irradiation and at UV 366 nm showed orange stain spots, and at UV 254 nm green stain spots. After being sprayed with 5% FeCl<sub>3</sub> reagent, the results before UV irradiation showed blackish green stains and at UV 366 nm showed black stains, and at UV 254 nm the results obtained yellowish green stains, so that the results of TLC with this Chinese betel leaf juice sample are suspected of containing tannin compounds. By using the mobile phase which is n-hexane: ethyl acetate (6:4).

The appearance of stains on the TLC plate before and after giving 5% FeCl<sub>3</sub> reagent with concentrated black and purple colors is positive for tannin compounds<sup>12</sup>. The n-hexane eluent is a non-polar solvent while ethyl acetate is a polar solvent. The eluent used is the best eluent and can show stains that are tannin compounds<sup>15</sup>.

b. Flavonoids

The TLC results obtained yellowish green stain spot results before UV irradiation and there are blue and black, fluorescent stain spots at UV 366 nm and then obtained a green stain spot at UV 254 nm. By using the mobile phase which is methanol: chloroform (1:9). The appearance of

stains on the TLC plate before and after giving ammonia vapor reagent with yellow, green and blue colors is positive for flavonoid compounds. Methanol eluent is a polar solvent while chloroform is a non-polar solvent, which is an eluent containing a solvent that can attract flavonoid compounds well<sup>12</sup>.

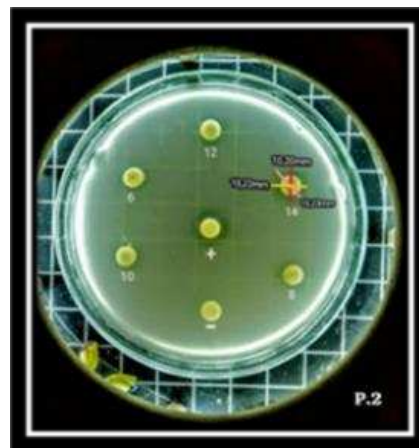
The results of the TLC test shown in Figures 4, 5, and 6, obtained the Rf value of each according to the distance traveled by the eluent. The alkaloid test had three stains with Rf values of 0.11, 0.15: 0.92. Stains with Rf values of 0.11 and 0.15 are suspected of having alkaloid compounds because they are included in the alkaloid test Rf range of 0.07-0.62<sup>16</sup>. The flavonoid test had six stains with Rf values of 0.35; 0.88; 0.92; 0.94; 0.94; 0.95. The stain with an Rf value of 0.35 is suspected to have flavonoid compounds because it is included in the flavonoid test Rf range of 0.2 - 0.75<sup>17</sup>. The tannin test has four stains with Rf values of 0.17; 0.25; 0.42; 0.72. The four Rf values are included in the Rf range of the tannin test, which is 0.07-0.77<sup>18</sup>.

Tests of TLC metabolite compounds in this study on samples of Chinese betel leaf juice have alkaloid, flavonoid and tannin compounds because they are included in the

Rf range in the resulting stain patches. The results of the Rf values are different because they are influenced by the eluent used, the eluent greatly affects the Rf value because it is the mobile phase on the TLC and because it is influenced by the level of polarity.

**Antibacterial Activity Test**

The results of the antibacterial activity test showed as follows:



**Figure 5.** Inhibition zone results of Chinese betel leaf juice on *Staphylococcus aureus* bacteria

**Table 1.** Results of Antibacterial Activity Testing on *Staphylococcus aureus* Bacteria.

| Concentration<br>(1) | Description<br>(2) | P.I<br>(3) | P.II<br>(4) | P.III<br>(5) | Average<br>(6) |
|----------------------|--------------------|------------|-------------|--------------|----------------|
| 6 µL/disc            |                    | 8,24       | 10,06       | 8,24         | 8,81           |
| 8 µL/disc            |                    | 10,04      | 9,14        | 9,22         | 9,46           |
| 10 µL/disc           | Chinese betel      | 9,86       | 10,11       | 9,28         | 9,75           |
| 12 µL/disc           | leaf juice         | 10         | 10,14       | 10,01        | 10,05          |
| 14 µL/disc           |                    | 10,06      | 10,21       | 10,11        | 10,12          |
| 10 µL/disc           | <i>Ampicillin</i>  | 10,13      | 10,28       | 10,03        | 10,14          |
| 6 µL/disc            | <i>Aquadest</i>    | 0          | 0           | 0            | 0              |

Based on the measurement of the average diameter of the inhibition zone, it is known that the concentration of 6µL/disc inhibits 8.81 mm, the concentration of 8µL/disc inhibits 9.46 mm, the concentration of 10µL/disc inhibits 9.75 mm, the concentration of 12µL/disc inhibits 10.05 mm, and the concentration of 14µL/disc inhibits 10.12 mm. The positive control concentration as a comparator inhibited 10.14 mm and in the negative control no zone of inhibition was formed. Based on the results of the average diameter of the inhibition zone formed at each concentration, it is categorized as being able to

inhibit the growth of *Staphylococcus aureus* with moderate intensity, namely the diameter of the inhibition zone of 5-10 mm, according to the Clinical and Laboratory Standard Institute (CLSI) antibacterial category<sup>19</sup>.

Alkaloids are special naturally occurring metabolites with nitrogen as a characteristic element present in their chemical structure. The rich biological potential of alkaloids is attributed to the different arrangement of atoms in their chemical structure<sup>20</sup>. And the mechanism of Alkaloid as an antibacterial is by interfering with the constituent components of peptidoglycan in

bacterial cells so that the cell wall layer is not formed intact and ultimately results in incomplete cell formation<sup>21</sup>.

Flavonoids are natural chemicals found in plants. Flavonoid compounds are distributed in plant parts such as seeds, flowers, leaves, roots and stems<sup>22</sup>. Flavonoids are phenolic compounds that are polar in nature<sup>23</sup>. As an antibacterial, it can be divided into 3 categories: inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting energy metabolism. Flavonoids will damage the cytoplasmic membrane and cause leakage of important metabolites, thus inactivating the bacterial enzyme system<sup>24</sup>. Tannins, on the other hand, can act as antibacterials by reacting with the cell membrane to inactivate enzymes and disable the function of genetic material. In addition, tannin compounds also have antibacterial mechanisms that can inhibit reverse transcriptase and DNA topoisomerase enzymes, thus preventing the formation of bacterial cells<sup>24</sup>.

The structure and composition of bacterial cells also play an important role in antibacterial mechanisms. *Staphylococcus aureus* bacteria are widespread flora that often cause disease and have the same potential as invasive pathogens based on coagulase synthesis. *Staphylococcus aureus* is one of the most resistant types of bacteria because it is based on bacteria that do not produce spores<sup>25</sup>.

**Table 3.** Mann Whitney Test Results

| Concentration | 6μL   | 8μL   | 10μL  | 12μL  | 14μL  | Positive | Negative |
|---------------|-------|-------|-------|-------|-------|----------|----------|
| (1)           | (2)   | (3)   | (4)   | (5)   | (6)   | (7)      | (8)      |
| 6μL           | -     |       |       |       |       |          |          |
| 8μL           | 0,513 | -     |       |       |       |          |          |
| 10μL          | 0,275 | 0,275 | -     |       |       |          |          |
| 12μL          | 0,275 | 0,275 | 0,376 | -     |       |          |          |
| 14μL          | 0,077 | 0,050 | 0,184 | 0,275 | -     |          |          |
| Positive      | 0,127 | 0,127 | 0,127 | 0,275 | 0,827 | -        |          |
| Negative      | 0,037 | 0,037 | 0,037 | 0,037 | 0,037 | 0,037    | -        |

Showing at concentration 1-2, namely (positive control and negative control) concentration 2-3, namely (negative control and 6μL/disc), concentration 2-4, namely (negative control and 8μL/disc), concentration 2-5, namely (negative control and 10μL/disc), concentration 2-6, namely (negative control and 12μL/disc), and concentration 2-7, namely (negative control and 14μL/disc) obtained a P value of 0.037 < 0.05, which means that there is

The results of data analysis using the Kruskal Wallis test in Table 5 show a P value or Asymptotic significance value of 0.035 < 0.05, which means that it is smaller than the specified significant value of 0.05 with a confidence level of 95%, it can be concluded that there is a significant difference or meaningful difference from each concentration, the effect of the inhibition zone of Chinese betel leaf juice on the growth of *S. aureus* bacteria that can inhibit bacterial growth affects the analysis.

**Table 2.** Kruskal Wallis Test Results

| Test Statistics <sup>a,b</sup> |                              |
|--------------------------------|------------------------------|
| Inhibitory Power               | <i>Staphylococcus aureus</i> |
| (1)                            | (2)                          |
| Chi-Square                     | 13.564                       |
| Df                             | 6                            |
| Asymp. Sig.                    | .035                         |

The results of the Kruskal Wallis test showed significant differences, so further tests were carried out using the Mann Whitney test to see the differences between each treatment group, in this case the concentration of Chinese betel leaf juice, namely, (6μL/disc, 8μL/disc, 10μL/disc, 12μL/disc and 14μL/disc), positive control (ampicillin 10μg/disc), and negative control.

a significant difference or there is a meaningful difference, meaning that the clear zone or inhibition formed looks different against *staphylococcus aureus* bacteria. It is said that there is a significant difference because the P value < 0.05.

While at concentrations 1-3, namely (positive control and 6μL/disc), concentrations 1-4, namely (positive control and 8μL/disc), concentrations 1-5, namely (positive control and

10 $\mu$ L/disc), obtained a P value of 0.127 > 0.05, which means that there is no significant difference or there is no meaningful difference, namely the clear zone or inhibition formed looks different against *Staphylococcus aureus* bacteria. At concentrations 1-6 namely (positive control and 12 $\mu$ L/disc), concentrations 3-5 namely (6 $\mu$ L/disc and 10 $\mu$ L/disc), concentrations 3-6 namely (6 $\mu$ L/disc and 12 $\mu$ L/disc), concentrations 4-5 namely (8 $\mu$ L/disc and 10 $\mu$ L/disc), concentrations 4-6 namely (8 $\mu$ L/disc and 12 $\mu$ L/disc), concentration 6-7, namely (12 $\mu$ L/disc and 14 $\mu$ L/disc), obtained a P value of 0.275 > 0.05, which means that there is no significant difference or there is no meaningful difference, namely the clear zone or inhibition formed looks different against *staphylococcus aureus* bacteria. And then concentrations 1-7, namely (positive control and 14 $\mu$ L/disc), obtained a P value of 0.827 > 0.05, meaning that there is no significant difference or there is no meaningful difference, namely the clear zone or inhibition formed looks different against *staphylococcus aureus* bacteria. Concentrations 3-4, namely (6 $\mu$ L/disc and 8 $\mu$ L/disc), obtained a P value of 0.513 > 0.05, meaning that there is no significant difference or there is no meaningful difference, namely the clear zone or inhibition formed looks different against *staphylococcus aureus* bacteria. Concentrations 3-7, namely (6 $\mu$ L/disc and 14 $\mu$ L/disc), obtained a P value of 0.077 > 0.05, concentrations 4-7, namely (8 $\mu$ L/disc and 14 $\mu$ L/disc), obtained a P value of 0.050 = 0.05, there is no significant difference or there is no meaningful difference, namely the clear zone or inhibition formed looks different against *Staphylococcus aureus* bacteria. Concentrations 5-6, namely (10 $\mu$ L/disc and 12 $\mu$ L/disc), obtained a P value of 0.376 > 0.05, meaning that there is no significant difference or there is no meaningful difference, namely the clear zone or inhibition formed looks different against *Staphylococcus aureus* bacteria. Concentrations 5-7 namely (10 $\mu$ L/disc and 14 $\mu$ L/disc) obtained a P value of 0.184 > 0.05, meaning that there is no significant difference or there is no meaningful difference, namely the clear zone or inhibition formed looks different against *Staphylococcus aureus* bacteria.

These bacteria can cause an infection characterized by inflammation that forms an abscess<sup>26</sup>. Infections caused such as acne, boils and wound infections on the skin, these bacteria

can cause more dangerous infections such as urinary tract infections, food poisoning and can cause death<sup>25</sup>. *Staphylococcus aureus* bacteria are bacteria that infect a lot, usually infections caused by these bacteria are found on the skin and nose<sup>27</sup>.

The many impacts on human health caused by *Staphylococcus aureus* bacteria make us more vigilant in finding ways to prevent and control the growth of these bacteria.

Based on the results of the study that the antibacterial activity test with *Staphylococcus aureus* bacteria. The activity formed starting from the concentration of 6 $\mu$ L/disc, 8 $\mu$ L/disc, 10 $\mu$ L/disc, 12 $\mu$ L/disc and 14 $\mu$ L/disc is moderate according to the category of inhibition zone diameter<sup>28</sup>.

#### 4. CONCLUSIONS

Based on the results of the study, it can be concluded that Chinese betel leaf juice shows antibacterial activity against *Staphylococcus aureus* bacteria. The results of the concentration treatment tested, namely 6 $\mu$ L/disc, 8 $\mu$ L/disc, 10 $\mu$ L/disc, 12 $\mu$ L/disc and 14 $\mu$ L/disc are categorized as moderate inhibition zone diameter, with the results of the *Kruskall Wallis* analysis test getting a significant value of 0.035 <  $\alpha$ .0.05 or a chi-square value of 13.564 > r.table (6) 12.592 with a confidence interval value of 95% and an error rate of 5%, meaning that there is a real or significant difference from each concentration carried out.

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