Inhibition of Juice Komba-Komba *Chromolaena odorata* Leaf Against Growth of Bacteria *Staphylococcus Aureus*

Reni Yunus, Muh.Hilman & Satya Darmayani

Department of Health Analyst, Poltekkes Kemenkes Kendari

**Abstract**

Nowadays many plants can be used as traditional medicines to overcome various diseases including infections, because the use of traditional medicines is relatively safer compared to drugs derived from chemicals. One of the local plants in Southeast Sulawesi that has been empirically used as a medicine for wounds is a race of plants. Komba-komba leaves *Chromolaena odorata* contain compounds alkaloid, flavonoids, tannins, phenols, triterpenoids, saponins and steroids which have their respective functions in inhibiting the growth of microorganisms or antibacterial. This study aims to determine the inhibition of komba-komba leaf extract *Chromolaena odorata* on the growth of bacteria *Staphylococcus aureus*. This type of research is experimental laboratories. The method used was agar diffusion with 5 treatment concentrations, namely komba-sheep leaf extract concentration *Chromolaena odorata* 20%, 40%, 60%, 80% and 100%, positive control (tetracycline) and negative control (aquadest) and repetition. The results showed that there was an inhibition zone of komba-komba leaf extract *Chromolaena odorata* on the growth of bacteria *Staphylococcus aureus* at a concentration of 20% at 2 mm, a concentration of 40% at 3.7 mm, a concentration of 60% at 4.7 mm, a concentration of 80% 6.2 mm and a concentration of 100% by 8 mm.

**Introduction**

Indonesia is a tropical country that has high biodiversity, making Indonesia has many plants used by the community as traditional medicine. In Indonesia there are around 30,000 types of plants and 7,000 of them have medicinal properties. Indonesia's diversity of biological resources ranks second after Brazil (Jumiarni & Komalasari, 2017).

All types of plants have long been targeted as new drug searches. The development of drug use, especially from plants to help improve public health. One of the benefits of using drugs from these plants is the Kirinyuh (plant*Chromolaena odorata*) which is used as an antibacterial (Awoyinka, 2007). Empirical studies on the local community of the Muna tribe show the use of this leaves as a wound healer. Kirinyuh leaves *Chromolaena odorata* in the local community of the Muna tribe are known as the leaves of "Komba-komba" (Jumiarni & Komalasari, 2017).
Indonesia is a tropical country so the prevalence of infectious diseases caused by bacteria is still high. One of the bacteria that infects wounds on the skin is the bacterium *Staphylococcus aureus*. Bacteria *Staphylococcus aureus* infects wounds, zits, ulcers and others. These wounds are usually treated with antibiotics which can inhibit inflammation and kill bacteria, for example, tetracycline, erythromycin, chloromhenicol, doxycycline and clindamycin. However, these drugs have side effects, namely irritating the skin to the wound or to zits, while the use of long-term antibiotics can also cause resistance and can cause organ damage and immuno hypersensitivity (Djajadisastra, 2009).

The prevalence of skin infections is increasing. Based on the results of the Nindya Nugerahdita 2009 study on the prevalence of skin disease and treatment in the Petamburan area of central Jakarta, the results showed that the prevalence of skin diseases of (47.57%) of 103 families observed in the Petamburan village in central Jakarta with the most skin diseases was caused by (71.43%) and the remainder is caused by bacteria of (28.57%).

In this era, many plants can be used as traditional medicines to treat various diseases including infections, because many people assume that the use of traditional medicines is relatively safer compared to drugs derived from chemicals. One of the plants that can be used as medicine is the komba-komba or kirinyuh plants. Leaves of komba-komba or kirinyuh *Chromolaena odorata* contain compounds alkaloid, flavonoids, tannins, phenols, triterpenoids, saponins and steroids which have their respective functions in inhibiting the growth of microorganisms or antibacterials (Dalimartha, 2000).

Previous research has shown that komba-komba leaves extract ointment or at a concentration of 20% has an effect in accelerating wound healing in laying hens (Rahman, 2017). In this study we used komba komba leaf extract without the addition of other chemical solvents such as ethanol or methanol to obtain active compounds. The reason researchers chose juice of komba komba leaf, without addition the chemical solvents, is that in its application as a natural antibiotic, people can make it easier. Based on the antibiotic content found in komba-komba leaf (*Choromolaena odorata*) which has efficacy as an antibacterial, researchers are interested in conducting research on "inhibition test of juice komba-komba leaf (*Choromolaena odorata*) on the growth of bacteria *Staphylococcus aureus*".

The purpose of this study was to determine the inhibition of juice komba-komba leaf *Chromolaena odorata* to the growth of *Staphylococcus aureus* and to determine the inhibition of juice komba-komba leaf *Chromolaena odorata* on the growth of bacteria *Staphylococcus aureus* at concentrations of 20%, 40%, 60 %, 80% and 100%.

**Materials and Methods**

This study was conducted in March-June 2018 at the Microbiology Laboratory of the Department of Health Analyst at the Poltekkes Kendari. The method used is experimental laboratories, using the One-shot Case Study design, namely research design with treatment of variables independent.

The inhibitory test of juice komba-komba *Chromolaena odorata* leaf using method agar diffusion. Data obtained by measuring the diameter of the inhibitory zone formed. Measurements were made twice with positive control tetracycline of 19 mm.
Tools and Materials
The tools used are digital balance, horn spoon, erlenmeyer, stirring rod, measuring pipette, ball filler, measuring cup, lamp spirits, three feet, wire mesh, incubator, atoclave, porcelain cup, test tube, tube rack, wire ose, tweezers, spray bottle, blender, filter paper, dropper pipette, ruler / ruler, funnel, beaker. The ingredients used were leaves of sheep *Chromolaena odorata*, antibiotics *tetracycline*, disc paper blank, media *nutrient agar* (NA), aquadest, filter paper, label paper, aluminum foil, pure culture of *Staphylococcus aureus*.

Pure Stock Making of *Staphylococcus aureus*
Bacteria The test bacteria used or bacteria to be purified is *Staphylococcus aureus*. The making of bacterial stock is carried out using ose then planted or inoculated by scraping on the media *NA* sloping and then incubated in an incubator temperature of 37°C for 1 x 24 hours.

Making Test Bacterial Suspensions.
The making of bacterial suspension was taken by test bacteria by using sterile ose wire and then suspended in 2 mL NaCl 0.9% in a sterile test tube and homogenized according to the standard Mc. Farland 0.5 which is characterized by the formation of turbidity after being suspended.

Sampling and Preparation of Samples
Leaves of komba - komba *Chromolaena odorata* taken are cleaned in washing with running water then stored at room temperature until dry after that the leaves are weighed as much as 500 grams then in a blender.

Making juice of Komba-Komba leaves *Chromolaena odorata*
Weighed 500 grams of leaves of the sheep *Chromolaena odorata* using digital scales and then blended and squeezed and filtered as much as 150 mL ago made in 5 variations of concentration, namely in variations in concentrations of 20%, 40%, 60%, 80%, and 100%.

Preparation of Tetracycline Antibiotic (Positive Control)
*Tetracycline* 250 mg created a concentration of 5% by weighing 0.5 grams of *tetracycline* then dissolved

Tests Antibacterial Activity
Antibacterial activity tested by using method *agar diffusion* (Disk Diffusion Method) of the Kirby-Bauer. The method is diffusion carried out by means of a 0.1 bacterial suspension mL spread on the surface of media *Nutrient Agar* (NA) by using ski gel then the *paper disc* is dipped in the leaf extract of the sheep *Chromolaena odorata* at each concentration of 20%, 40%, 60, 80% and 100% using tweezers stored on the surface of media *Nutrient Agar* (NA) then labeled using label paper. Then a positive control was made using *tetracycline paper disc* dipped in *tetracycline* for positive control and planted on the surface of the media *Nutrient Agar* (NA) and then incubated for 1x24 hours temperature 37°C.

Results and Discussion
This study shows that the juice of the leaf Komba-komba *Chromolaena odorata* has an inhibitory effect on *Staphylococcus aureus*. Testing of inhibition was carried out twice repetition, the first major inhibition was found at a concentration of 100% with a diameter of 10.5 mm and the smallest inhibition was found at a concentration of 20% with a diameter of
1.5 mm while in the repetition of both power the biggest inhibition was at a concentration of 100% with a diameter of 5.5 mm and the smallest inhibition was found at a concentration of 20% with a diameter of 2.5 mm. For the results of research the inhibitory zone can be seen more clearly in figure 1 and table 1 below.

![Figure 1. inhibition zone formed after incubation](image)

**Table 1. Results Measurement of inhibition zones juice of komba-komba leaf *Chromolaena odorata* on the growth of bacteria *Staphylococcus aureus***

<table>
<thead>
<tr>
<th>No</th>
<th>Concentration</th>
<th>Observation time</th>
<th>Average diameter of inhibition zone</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 %</td>
<td>24 hours</td>
<td>2 mm</td>
<td>Resistent</td>
</tr>
<tr>
<td>2</td>
<td>40 %</td>
<td>24 hours</td>
<td>3.7 mm</td>
<td>Resistent</td>
</tr>
<tr>
<td>3</td>
<td>60 %</td>
<td>24 hours</td>
<td>4.7 mm</td>
<td>Resistent</td>
</tr>
<tr>
<td>4</td>
<td>80 %</td>
<td>24 hours</td>
<td>6.2 mm</td>
<td>Resistent</td>
</tr>
<tr>
<td>5</td>
<td>100 %</td>
<td>24 hours</td>
<td>8 mm</td>
<td>Resistent</td>
</tr>
<tr>
<td>6</td>
<td>Control (+)</td>
<td>24 hours</td>
<td>19 mm</td>
<td>Sensitive</td>
</tr>
<tr>
<td>7</td>
<td>Control (-)</td>
<td>24 hours</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Discussion**

In the test of inhibitory power of juice of komba-komba *Chromolaena odorata* leaf which will be tested on bacteria *Staphylococcus aureus* with using method *agar diffusion* or scatter method with juice of komba-komba leaf testing *Chromolaena odorata* made in 5 variations of concentration, namely concentrations of 20%, 40%, 60%, 80% and 100% carried out in the Laboratory of Health Polytechnic Kendari Department of Health Analyst.

The testing of inhibitory power of juice komba-komba leaf *Chromolaena odorata* on the growth of bacteria was *Staphylococcus aureus* carried out in several stages, starting from the leaf selection stage to testing the bacterial inhibition. The stage of leaf selection is done by selecting leaves that are still young and taken by manual then carried out until the stage of making the concentration for testing the inhibitory power.

Testing the inhibition of the growth of *Staphylococcus aureus* by using the method *diffusion to be* incubated for 1 x 24 hours in an incubator with inhibition zones was characterized by the formation of clear areas around the *paper disc*, using drugs *tetracycline* as a positive control and *aquadest* as a negative control.

The inhibitory power of juice komba-komba *Chromolaena odorata* leaf at a concentration of 20% inhibition zone formed an average of 2 mm. The concentration of 40% of the inhibition zone formed 3.7 mm. The concentration of 60% of the inhibition zone formed 4.7 mm. Concentration of 80% inhibition zone 6.2 mm. The concentration of 100% of the inhibition zone formed is 8 mm. So that from the 5 concentrations formed a clear area around the *paper disc* which is called the inhibitory zone. The inhibitory zone formed is still categorized as resistant (weak) because of the large inhibition zone formed less than 12 mm but at a concentration of 100% in this test although it is included in the category of resistance.
but effective in inhibiting the growth of *Staphylococcus aureus* because it has the greatest inhibitory power testing this obstacle.

This is in line with the research of Purwati and Undri Rastuti (2009) explaining *ethyl acetate* leaf *Eupatorium odoratum* extracts based on screening of secondary metabolites containing *flavonoids*, with secondary metabolites in extract of *ethyl acetate* leaves *Eupatorium odoratum* has antioxidant activity with a sequence of inhibitory activities of 0.15 % (b / v). *Flavonoids* denature bacterial cell proteins and can damage bacterial cytoplasmic membranes so that they can inhibit the growth of bacteria *Staphylococcus aureus* in testing the inhibitory power.

In another study (Rahman, 2017) also used *Chromolaena odorata* leaf extract incisive for wound healing in laying hens, which in their study showed that the effective concentration of deep wound healing in laying hens was at a concentration of 20%.

The previous research by Vital and Rivera (2009) in his research carried out a test of the antimicrobial activity of leaf extract *Chromolaena odorata*, the results showed positive for bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhimurium*. Previous research also showed the effectiveness of extract of ethanol leaves of *Chromolaena odorata* in wound healing in male mice (Afrianti, 2010).

**Conclusions**

The research show that juice of leaf komba-komba *Chromolaena odorata* has inhibition power to the growth of bacteria *Staphylococcus aureus*.

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