

ANTIBACTERI ACTIVITY TEST OF ETANOL EXTRACT OF SEMBUNG LEAVES (*Blumea balsamifera*) ON THE GROWTH OF *Staphylococcus aureus* BACTERIES BY USING THE CAKRAM METHODS

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ABSTRACT

Staphylococcus aureus is a harmful type of bacteria. Infectious diseases caused by these bacteria are boils, acne, and skin infection. Sembung leaves (*Blumea balsamifera*) have secondary metabolite compounds such as flavonoids, alkaloids, tannins and steroids that have potential as antibacterials. The purpose of this study was to determine the antibacterial activity of ethanol extract of Sembung leaves (*Blumea balsamifera*) with concentrations of 10%, 25%, and 50% against the growth of *Staphylococcus aureus* bacteria with the disc method. This type of research is Quasy experimental antibacterial tests carried out by making paper disks that are dripped with extracts with concentrations of 10%, 25%, 50% and to determine the concentration with the greatest inhibitory effect. Extraction was carried out by maceration method using 96% ethanol solvent. Inhibition testing of ethanol extract of Sembung leaf (*Blumea balsamifera*) was done by disc method. Positive control used chloramphenicol. The results showed the average number of inhibition zones of 10% concentration (11.6) mm, 25% concentration (13.3) mm, 50% concentration (14.6) mm, the positive control produced an inhibition zone of 20 mm categorized as very strong, while the negative control did not form an inhibition zone. Based on the research that has been done, it can be concluded that the inhibition at a concentration of 50% gives the greatest inhibitory effect.

Keywords: *Staphylococcus aureus*, Sembung leaf (*Blumea balsamifera*), Antibacterial, Maceration, Disc method

INTRODUCTION

The people of Southeast Sulawesi often use sembung leaves (*Blumea balsamifera*) as a traditional herbal remedy to treat various ailments, including acid reflux, headaches, fever, diarrhea, diabetes, improve blood circulation, kill or eliminate germs (bactericidal), stimulate sweating (diphoretic), thin mucus (expectorant), treat menstrual pain, and treat postpartum wounds. Sembung leaves are a medicinal plant developed as an antibacterial (Amalia, Sari, and Nursanty 2017).

Sembung leaves (*Blumea balsamifera*) are widely cultivated throughout East and Southeast Asia, from India to Myanmar, Indonesia and China, southern China, Taiwan, and the Philippines. This plant grows naturally on the sides of roads, in highlands, and is filled with reeds, in fields where livestock are grazed, in bushes and in forests, for example bamboo or teak forests, as well as in wet places and on riverbanks, at an altitude of 2200 m above sea level, growing in groups under the sun, is considered a pest and is very easy to eradicate.

The gram-positive bacterium *Staphylococcus aureus* is responsible for an increasing number of illnesses and deaths each year. Many diseases are caused by this bacterium, which colonizes human skin and nose (Rianti, Tania, and Listyawati 2022). Gram-positive *Staphylococcus aureus* bacteria, which can grow singly or in pairs, have a diameter of 0.5 to 1.5 μm and resemble irregular grapes. These bacteria can cause infections with characteristic symptoms (Nur Khairunnisa et al. 2023). Boils, acne, and skin infections are some of the infectious disorders caused by the dangerous *Staphylococcus aureus* bacteria. Boils are skin infections that affect the sebaceous glands, sweat glands, and hair follicles (Kulla and Herrani 2022).

According to Ameliana (2022), Sembung leaves contain active compounds such as alkaloids, flavonoids, tannins, and steroids, which have antibacterial properties. Alkaloids function by disrupting bacterial metabolism and DNA structure. Flavonoids function by damaging bacterial cell membranes and inhibiting DNA activity. Meanwhile, tannins cause damage to cell membranes through their toxic properties and form complexes with bacterial proteins to inhibit their growth. Steroids function to damage bacterial membrane lipids. This study shows that the ethanol extract of Sembung leaves has significant inhibitory power against bacteria, this indicates that Sembung leaves have great potential to be used as a natural ingredient for treating infections caused by bacteria (Ameliana et al. 2022). Based on Amalia's research (2017) the results showed that the activity test of Sembung leaf extract at a concentration of 10% was 10.83 mm, a concentration of 20% was 12.41 mm, it was concluded that the inhibition zone was categorized as moderate (Amalia, Sari, and Nursanty 2017). Furthermore, Sa'adah (2024) conducted research on the ethanol extract of Sembung leaves with N-Hexane fractions and ethyl acetate fractions using the disc method, and used 1% clindamycin as a comparator. The results showed that the ethanol extract of Sembung leaves could inhibit the growth of *Staphylococcus epidermidis* bacteria at a concentration of 10% by 8.1 mm, a concentration of 25% by 9.13 mm, and a concentration of 50% by 10.1 mm. So it was concluded that the higher the concentration, the stronger the inhibition zone (Sa'adah, Pristanti, and Warnida 2024).

METHOD

Types of research

The research design used was a quasi-experimental study, meaning that this design has controls, but cannot fully control external variables that influence the experiment. This study used the disc method. The subject was Ethanol extract of Sembung (*Blumea balsamifera*) leaves and the object was the antibacterial activity of ethanol extract of Sembung (*Blumea balsamifera*) leaves against the growth of *Staphylococcus aureus* bacteria.

Equipment and Materials

The equipment required for this study is: Blank paper disks, Petri dishes, Ose needles, 250 ml Pyrex measuring flasks, 250 ml Pyrex Erlenmeyer flasks, stirring rods, aluminum foil, markers, Bunsen burners, matches, cotton swabs, tweezers, vials, autoclave (Hiramaya), knife, ruler, incubator (Labnet), 250 ml Pyrex beakers, analytical balance (Kern), rotary evaporator (Janke & Kunkel RV 06-ML), blender, test tubes (Iwaki), separating funnels (Pyrex), tissue, and test tube rack.

The materials used in this study were Sembung leaves (*Blumea balsamifera*), *Staphylococcus aureus* bacteria, Mueller Hinton Agar (MHA), chloramphenicol as a positive control, and 96% ethanol.

Ways of working

The leaves used are leaves from the sembung plant (*Blumea balsamifera*) that are neither too young nor too old, green in color and without holes. They are then powdered and macerated. Test the extract's activity against the growth of *Staphylococcus aureus* bacteria using the disc method.

- Prepare four petri dishes with a diameter of 9 cm.
- Take the discs containing the extract one by one using sterilized tweezers.
- Place them on the surface of the Mueller Hinton Agar medium inoculated with *Staphylococcus aureus* bacteria, spacing them approximately 1.5–2 cm apart.
- Mark the discs on the solvent dish where the discs were inoculated.
- Incubate for 18 hours at 37°C (Wijaya 2018).
- Create positive and negative controls.

Data analysis.

Data can be obtained from measuring the diameter of the bacterial inhibition zone formed. The inhibition zone formed around the media is measured in vertical and horizontal diameter in millimeters (mm) using a vernier caliper/ruler.

RESULTS AND DISCUSSION

The antibacterial activity test aims to determine the inhibition zone of the ethanol extract of Sembung leaves (*Blumea balsamifera*) as an antibacterial against *Staphylococcus aureus* bacteria using concentrations of 10%, 25%, 50%. The control used in this study was a negative control using distilled water and a positive control using the antibiotic disk chloramphenicol.

Table 1 Data on the inhibitory power of ethanol extract of Sembung leaves

Extract Concentration %	Inhibition zone (mm)			Average	Category
	P1	P2	P3		
10%	11 mm	12 mm	12 mm	11,6 mm	Strong
25%	14 mm	13 mm	13 mm	13,3 mm	Strong
50%	15 mm	14 mm	15 mm	14,6 mm	Strong
Cloramphenicol(+)	20 mm				Very Strong
Aquadest(-)	0				-

This study used Sembung leaves (*Blumea balsamifera*) collected from Tarikan village, Kumpeh Ulu sub-district, Muaro Jambi regency. The preparation of the medicinal plant was carried out by collecting fresh leaves in the morning, leaves that were not perforated, not eaten by insects, or contaminated with animal feces. The purpose of selecting leaves in the morning was to obtain fresh leaves, because at that time the plant was not directly exposed to the sun's heat, which can cause the evaporation of active compounds such as flavonoids and alkaloids. Furthermore, the risk of contamination by insects was also lower in the morning compared to the afternoon or evening. Perforated leaves have the potential to carry foreign microorganisms that can affect the extraction process, as well as reduce the quality and antibacterial activity of the resulting extract. The next stage was wet sorting, the purpose of this sorting was to maintain the purity of the medicinal plant and prevent microorganism contamination. After wet sorting, washing was carried out with running water to remove dust, soil, and microorganisms that might be attached to the leaf surface. The next stage was the chopping stage, which involved cutting the leaves into small pieces to speed up the drying process and help the solvent reach the active compounds optimally during extraction. In the drying process, the simplicia is covered with a black cloth, covering it with this black cloth aims to protect the active compounds from degradation due to direct exposure to sunlight which can damage the chemical structure of important compounds such as flavonoids and alkaloids. Next, a dry sorting process is carried out which aims to separate materials that are damaged or discolored during the drying process. After that, the results of drying shrinkage are obtained by comparing the weight of the dry simplicia and the weight of the fresh simplicia of 9.5%, which is included in the good category according to the maximum water content standard of simplicia of no more than 10% (herbal pharmacopoeia 2023). The next stage is refining using a blender so that the simplicia will be smooth. This refining process aims to reduce the particle size and increase the surface area so that during the extraction process, with a large surface area, the solvent will enter the simplicia and can draw all the active substances in it. After that, it is sieved with a no. 40 sieve which aims to obtain powder particles with a uniform size. The next stage is the preparation of the extract. The maceration method is used to make Sembung (*Blumea balsamifera*) leaf extract. Maceration is a simple extraction method, a technique for extracting simple herbs using a solvent, which involves several steps of soaking and stirring at room temperature (Athallah and Sugesti 2020). The solvent used is 96% ethanol with a ratio of 1:10. This solvent can effectively extract polar and semi-polar compounds, such as flavonoids, tannins, and other phenolic compounds. In addition, the high ethanol concentration facilitates penetration into plant tissue, thus accelerating the dissolution of active compounds. Ethanol is also volatile, thus facilitating the evaporation process after extraction (Septiningrum, Ariastuti, and Ahwan 2024). The wet weight of Sembung leaves used was 1 kg, then made into a simple powder weighing 100 grams. For extraction, maceration was used with 1 liter of 96% ethanol solvent. Soaked for 3 days, filtered using filter paper, the filtered extract was concentrated using a rotary evaporator at a temperature of 60 °C. The evaporated extract was then thickened in a water bath to obtain a thick extract. After obtaining the thick extract, the yield was calculated, resulting in a yield of 15.2%, which is considered good; a good yield of thick extract is greater than 10% (Herbal Pharmacopoeia 2023).

After obtaining the thick extract, secondary metabolite identification tests were conducted on the thick extract of Sembung (*Blumea balsamifera*) leaves. The aim was to determine the compounds present in Sembung (*Blumea balsamifera*) leaves, especially those that can inhibit bacterial growth. Sembung leaves contain active compounds such as alkaloids, flavonoids, tannins, and steroids, which have antibacterial properties (Ameliana et al. 2022). Based on the secondary metabolite identification tests performed, the ethanol extract of Sembung (*Blumea balsamifera*) leaves showed positive flavonoids, alkaloids, tannins, and saponins. The presence of flavonoids results in a red color after adding Mg powder and then adding 2N HCl. The

presence of alkaloids results in a brick-red precipitate after adding Dragendorff's reagent. The presence of tannins results in a blackish-green color after adding FeCl_3 . The presence of saponins is indicated by the formation of foam. The sample is placed in a test tube, then added to distilled water and heated in a water bath at 100°C for 15 minutes. After cooling, shake vigorously vertically. The presence of steroids forms a red ring after adding chloroform and concentrated H_2SO_4 , but in this study, the steroid test was negative. The unexpected cause was an unclean test tube. The presence of terpenoids produces a purplish-red color, but the test did not produce a positive result; the terpenoids are greenish-brown. The unexpected cause is an unclean dropper plate or insufficient reagent input.

To determine the inhibitory power of the ethanol extract of Sembung (*Blumea balsamifera*) leaves, a disc diffusion test was conducted. because the disc method is one of the effective methods for testing antibacterial activity due to its simple procedure, and produces results in the form of inhibition zones that can be easily measured, making it suitable for testing antimicrobials (Nurhayati, Yahdiyani, and Hidayatulloh 2020). Then proceed with making the seed media. The preparation is carried out aseptically, so that the media remains sterile. The agar media is weighed then dissolved with distilled water and boiled on a hotplate, after which the petri dish is sterilized in an autoclave at a temperature of 121°C for 15 minutes to avoid contamination. After the media has cooled, it is poured into 4 petri dishes. The media used is MHA (Mueller Hinton Agar), MHA is used because it has good nutritional content for most bacterial cultures, and also MHA is neutral, so it does not affect the working method of the antibacterial test (Utomo et al. 2018). To make a suspension of cultured bacteria, 1-2 loops were taken and suspended in a 0.9% NaCl solution to obtain turbidity according to the Mc Farland solution turbidity standard with a standard of 0.5 (Sa'adah, Pristanti, and Warnida 2024). This bacterial suspension was then applied evenly to the entire surface of the MHA medium using sterile cotton wool. Before being attached to the inoculated surface of the MHA medium, each disc was first soaked in the extract for 15 minutes according to the specified concentration. Afterward, the discs were implanted using sterile tweezers onto the surface of the medium at concentrations of 10%, 25%, and 50%. The purpose of these various concentrations was to determine the optimal antibacterial concentration. The antibiotic chloramphenicol was used as a positive control and distilled water as a negative control, with three repetitions.

Chloramphenicol was used as a positive control because it is a broad-spectrum antibiotic that attacks bacterial protein synthesis, thus inhibiting the growth of both gram-positive and gram-negative bacteria (Syafriana et al. 2020). The bacteria used in this study was *Staphylococcus aureus*, as this bacterium is one of the causes of boils, acne, and impetigo. Boils are skin infections that occur in the hair follicles, sebaceous glands, and sweat glands (Kulla and Herrani 2022). Table 4.5 shows the results of the inhibitory power test of the ethanol extract of Sembung leaves (*Blumea balsamifera*). The inhibition zone with various concentrations has an effect on the growth of *Staphylococcus aureus* bacteria. At a concentration of 10%, it is 11.6 mm, at a concentration of 25% it is 13.3 mm, and at a concentration of 50% it is 14.6 mm. Inhibition zones with a diameter of ≤ 5 mm are categorized as weak, inhibition zones with a diameter of 6-10 mm are categorized as moderate, inhibition zones with a diameter of 11-20 mm are categorized as strong, inhibition zones with a diameter of ≥ 21 mm are categorized as very strong (Andini 2020). Where the higher the concentration of Sembung leaf ethanol extract (*Blumea balsamifera*) used, the larger the diameter of the inhibition zone, based on the standard interpretation of the inhibition zone in the Antibiotic Clinical Laboratory Standards Institute (CLSI), Chloramphenicol is categorized as inhibiting if it has an inhibition zone diameter of ≥ 17 mm, intermediate 14-16 mm, resistant ≤ 13 mm (Jampur et al., 2024). In this study, chloramphenicol antibiotics were used as a positive control with the results of antibacterial activity testing with an inhibitory power of 20 mm against *Staphylococcus aureus* bacteria in the very strong category Aquadest was used as a negative control, and the results showed no inhibition zone formation. Aquadest was chosen as a negative control because it does not contain active substances capable of inhibiting bacterial growth. This ensures that the inhibitory effect originates from the extract or antibiotic, not the medium or solvent (Nadlif and Walid 2024).

This study aligns with research by Sa'adah, Pristanti, and Warnida 2024. This study used ethanol extract of Sembung (*Blumea balsamifera*) leaves, but the bacteria *Staphylococcus epidermidis* was inhibited at a concentration of 10%, 9.13 mm at a concentration of 25%, and 10.1 mm at a concentration of 50%. The higher the concentration of ethanol extract of Sembung (*Blumea balsamifera*) leaves, the larger the inhibition zone formed. The difference in the diameter of the bacterial growth inhibition zone at each concentration is due to the dilution of each concentration. Research results demonstrate that ethanol extract of Sembung (*Blumea balsamifera*) leaves can inhibit the growth of *Staphylococcus aureus* bacteria as an antibacterial. Therefore, ethanol extract of Sembung (*Blumea balsamifera*) leaves can be developed as a natural antibacterial drug.

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