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**PHYTOCHEMICAL PROFILING AND ANTIBACTERIAL ACTIVITY OF
 SINGKAI (*PERONEMA CANESCENS*) LEAF EXTRACT AGAINST
*STAPHYLOCOCCUS AUREUS***

**Noor Muthmainah¹⁾, Eko Suhartono^{2,3)*}, Gusti Muhammad Perdana Putera⁴⁾, Dona Marisa⁵⁾, Mashuri⁶⁾,
 Lisda Hayatie¹⁾, Iskandar⁷⁾, Syaifullah Akbar Pradito⁸⁾**

¹⁾Department of Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universitas
 Lambung Mangkurat, Banjarmasin, South Kalimantan – Indonesia

²⁾Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Health Sciences, Universitas
 Lambung Mangkurat, Banjarbaru, South Kalimantan – Indonesia

³⁾Health Research Center for Wetland Faculty of Medicine and Health Sciences, Universitas Lambung
 Mangkurat, Banjarbaru, South Kalimantan – Indonesia

⁴⁾Faculty of Dentistry, Universitas Lambung Mangkurat, Banjarmasin, South Kalimantan – Indonesia

⁵⁾Department of Biomedic, Faculty of Medicine and Health Sciences, Lambung Mangkurat University,
 Banjarmasin, South Kalimantan – Indonesia

⁶⁾Department of Radiology, Faculty of Medicine and Health Sciences, Universitas Lambung Mangkurat,
 Banjarmasin, South Kalimantan – Indonesia

⁷⁾Public Health Study Program, Undergraduate Program, Faculty of Medicine and Health Sciences, Universitas
 Lambung Mangkurat, Banjarbaru, Indonesia

⁸⁾Medical Graduate, Faculty of Medicine and Health Sciences, Universitas
 Lambung Mangkurat, Banjarmasin, South Kalimantan – Indonesia

ABSTRACT

Background: *Staphylococcus aureus* is an opportunistic Gram-positive bacterium that can colonize the oral cavity and cause various infections such as oral abscesses, stomatitis, and post-dental procedure infections. The increasing problem of antibiotic resistance has encouraged the exploration of medicinal plants as alternative antibacterial agents. Sungkai (*Peronema canescens*) leaves are traditionally used by the Dayak Meratus community as herbal medicine and are known to contain bioactive compounds with potential antibacterial activity. **Purpose:** This study aimed to analyse the phytochemical content and antibacterial activity of sungkai leaf extract against *Staphylococcus aureus*. **Methods:** This research used a true experimental design with a post-test-only control group design. Methanol extract of sungkai leaves was prepared using the maceration method. Phytochemical analysis included determination of flavonoid, alkaloid, and steroid contents using spectrophotometric and gravimetric methods. Antibacterial activity was tested using the disc diffusion method on Mueller Hinton Agar against *S. aureus* at concentrations of 25%, 50%, 75%, and 100%. Clindamycin was used as a positive control and sterile distilled water as a negative control. **Results:** Phytochemical analysis showed that sungkai leaf extract contained flavonoids (85.47 mg/mL QE), steroids (26.797%), and alkaloids (18.436%). The antibacterial test showed inhibition zones that increased with extract concentration, namely 10.19 ± 0.23 mm (25%), 12.89 ± 0.43 mm (50%), 14.80 ± 0.41 mm (75%), and 17.72 ± 0.57 mm (100%). **Conclusion** Sungkai leaf extract contains several bioactive compounds and demonstrates antibacterial activity against *Staphylococcus aureus*, indicating its potential as a natural antibacterial agent.

Keywords: Antibacterial Activity, Phytochemical Analysis, *Staphylococcus aureus*, Sungkai Leaf Extract.

Correspondence: Eko Suhartono, Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Health Science, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia, Email: esuhartono@ulm.ac.id, Phone number: +62081251126368

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a Gram-positive coccus bacterium that is opportunistic and can be found in the oral cavity even though it is not part of the normal flora.¹ This type of bacteria can colonize the oral mucosa, dental plaque, saliva, and the surface of dental prostheses or implants. *S. aureus* plays a role in several pathological conditions such as oral abscesses, soft tissue infections, stomatitis, and post-dental procedure infections such as

tooth extraction and implant placement.² Its ability to form biofilms and produce various virulence factors allows this bacterium to survive on tooth surfaces and oral tissues, thereby potentially exacerbating odontogenic infections and increasing the risk of systemic complications in certain patients.³

Currently, treatment of *S. aureus* bacterial infections requires antibiotics such as cloxacillin, cephalosporin, clindamycin, and amoxicillin combined with clavulanic acid.^{4,5} However, inappropriate use of antibiotics can cause bacterial resistance, disrupt the balance of oral microbiota, and reduce the effectiveness of therapy for subsequent infections. In addition, excessive use can also trigger side effects such as allergic reactions, gastrointestinal disorders, and an increased risk of other opportunistic infections. In this regard, efforts are needed to explore plants that have potential as antibiotics. One such plant is sungkai leaves (*Paronema canescens*).

Research by Elsi et al.⁶ states that the Dayak Meratus indigenous people use the mucilage in the bark as a traditional medicine to cure toothache. The study also revealed that methanol extract of sungkai leaves contains alkaloids, flavonoids, phenols, triterpenoids, and tannins. These compounds are secondary metabolites that possess antibacterial properties.⁶ Meanwhile, research by Mashuri et al.⁷ also found that sungkai leaf extract can protect erythrocytes from UV exposure. The study also mentions that sungkai leaf ethanol extract contains D-limonene, which can act as an antioxidant. Other studies also mention that D-limonene is a monoterpene compound that has antibacterial activity. This compound works by damaging the bacterial cell membrane, disrupting membrane permeability, and inhibiting microbial metabolism. Several studies show that D-limonene is effective in inhibiting the growth of Gram-positive bacteria, including *Staphylococcus aureus*, thus having potential as a natural antibacterial agent.^{8,9}

However, previous studies have not revealed the phytochemical content of sungkai leaves, such as steroids, alkaloids, and flavonoids, which also act as antibacterials. In this regard, this study will reveal the phytochemical analysis of sungkai leaf extract as an antibacterial agent. Thus, sungkai leaves have the potential to be used as an alternative source of antibacterial compounds to support the development of herbal medicines and natural-based health products in the future.

MATERIALS AND METHODS

The method used in this study was a true experimental design with a Posttest-Only with Control Group Design. The treatment groups tested were sungkai leaf extract preparations at concentrations of 25%, 50%, 75%, and 100%. Clindamycin was used as the positive control and sterile distilled water as the negative control. The parameter observed was the measurable inhibition zone from the *S. aureus* antibacterial activity test on Mueller Hinton Agar (MHA) media after receiving the test treatment.¹⁰ The number of repetitions for each group was 3 times, obtained from the calculation results using the Federer formula. This study has been approved by the Research Ethics Committee under Number 210/KEPK-FKIK ULM/EC/X/2024, dated October 24, 2024.

Methanol Extract of Sungkai Leaves

The concept of methanol extraction of sungkai leaves (*Peronema canescens*) is to soak dried leaf powder in methanol solvent at room temperature for several days while stirring occasionally. Methanol dissolves active compounds such as flavonoids, alkaloids, and phenolics. After that, the solution is filtered and evaporated to obtain a thick extract containing secondary metabolites. The maceration method follows previous research.⁷

Determination of Flavonoid Content

Flavonoid measurement using the $AlCl_3$ (aluminum chloride) method is based on the formation of complexes between aluminum ions and flavonoid hydroxyl groups. These complexes produce a yellow color that can be measured using a spectrophotometer at a specific wavelength. The color intensity is proportional to the flavonoid concentration, usually expressed as quercetin equivalents in the extract sample. The flavonoid measurement procedure follows the method described by Nugroho et al.¹¹

Determination of Alkaloid Content

Alkaloid measurement using the Harborne method is based on the principle of gravimetry, which is a measurement based on precipitation. The method of measuring alkaloids using the previous method works as follows.¹¹

Determination of Steroid Content

Steroid measurements in plant extracts are generally performed using colorimetric methods with reagents such as Liebermann–Burchard. Steroids react with these reagents to form green or blue color changes. The color intensity is then measured using a spectrophotometer at a specific wavelength. The absorbance value obtained is proportional to the steroid concentration in the extract sample. The steroid measurement method follows the previous method.¹²

Determination of Antibacterial Activity of Sungkai Leaf Extract

The antibacterial activity of sungkai leaf extract was tested using the disc diffusion method.¹³ The test bacterial suspension was prepared in sterile physiological NaCl and adjusted to a McFarland standard of 0.5. The bacterial suspension was then evenly inoculated onto the surface of sterile Mueller Hinton Agar medium. Sterile discs that had been dripped with sungkai leaf extract (at concentrations of 25%, 50%, 75%, and 100%) were placed on the surface of the medium. Clindamycin was used as a positive control, while the extract solvent was

used as a negative control. All dishes were incubated at 37°C for 24 hours. Antibacterial activity was determined based on the diameter of the inhibition zone formed around the disc, then measured in millimeters.

Data Analysis

Phytochemical content is expressed as mean and standard deviation. The normality test uses the Shapiro-Wilk test, because the number of samples tested is small (<50), and the homogeneity of variance is tested using Levene's test. Meanwhile, bacterial activity testing uses ANOVA testing followed by post hoc testing.

RESULTS

Based on phytochemical testing, sungkai leaf extract contains the following phytochemical compounds (Figure 1). The results of phytochemical analysis of sungkai leaf extract show that the extract contains several major secondary metabolites, namely flavonoids, steroids, and alkaloids. Based on the graph, the flavonoid content is the highest at 85.47 mg/mL QE. The high flavonoid content indicates that sungkai leaf extract has the potential for strong biological activity, such as antioxidant, anti-inflammatory, and antibacterial activity, because flavonoids are known to be able to capture free radicals and inhibit the growth of microorganisms.

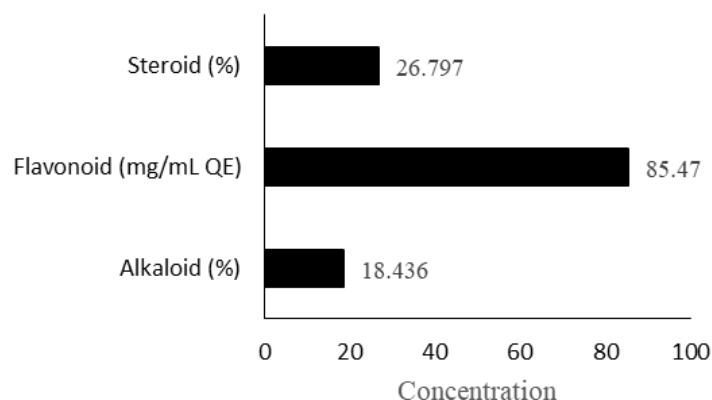


Figure 1. Average phytochemical content of sungkai leaf extract

In addition to flavonoids, sungkai leaf extract also contains 26.797% steroids and 18.436% alkaloids. Steroid content can play a role in anti-inflammatory and antimicrobial activities, while alkaloids are known to have various pharmacological activities such as antibacterial and antimalarial. Although their levels are lower than flavonoids, the presence of these two compounds indicates that sungkai leaf extract has a diverse composition of secondary metabolites that have the potential to provide synergistic biological effects. Meanwhile, the results of antibacterial testing of sungkai leaf extract are presented in Table 1 and figure 2.

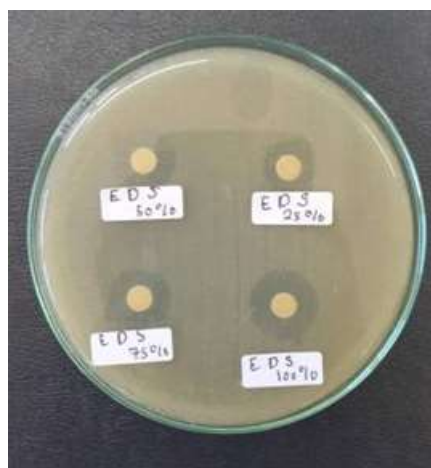


Figure 2. Antibacterial activity of sungkai leaf extract

Table 1. Sungkai leaf extract showed antibacterial activity

Replication	Sungkai leaf extract				Clindamycin 2 µg
	25%	50%	75%	100%	
1	10.15	13.46	14.40	18.28	21.81
2	9.93	12.42	14.64	16.94	22.41
3	10.50	12.8	15.37	17.95	21.72
Average ± SD	10.19 ± 0.23	12.89 ± 0.43	14.80 ± 0.41	17.72 ± 0.57	21.98 ± 0.31

Based on Table 1, sungkai leaf extract showed antibacterial activity characterized by the formation of inhibition zones at various concentrations. The average diameter of the inhibition zone increased with increasing extract concentration, namely 10.19 ± 0.23 mm at a concentration of 25%, 12.89 ± 0.43 mm at 50%, 14.80 ± 0.41 mm at 75%, and 17.72 ± 0.57 mm at 100%. This increase in the diameter of the inhibition zone indicates that the higher the concentration of sungkai leaf extract, the stronger the extract's ability to inhibit bacterial growth ($p=0.03$; $p<0.05$).

When compared to the positive control of clindamycin 2 μ g, which produced an inhibition zone diameter of 21.98 ± 0.31 mm, the antibacterial activity of sungkai leaf extract was still lower. However, at the highest concentration (100%), the extract still showed a fairly strong inhibitory effect. This antibacterial activity is likely influenced by the content of secondary metabolites such as flavonoids, alkaloids, and steroids found in sungkai leaf extract, which are known to have the ability to disrupt the structure of cell walls and bacterial metabolism.

DISCUSSION

Sungkai leaf extract (*Peronema canescens*) is known to contain various secondary metabolites that play an important role in the biological activity of plants. Based on phytochemical analysis, the main compounds identified in sungkai leaf extract include flavonoids, alkaloids, and steroids. These compounds are bioactive components commonly found in medicinal plants and are often associated with various pharmacological activities such as antioxidant, antibacterial, anti-inflammatory, and immunomodulatory activities.

Flavonoids are one of the dominant compounds in sungkai leaf extract. These compounds belong to the polyphenol group, which has a high ability to capture free radicals and protect cells from oxidative damage. In addition to their antioxidant properties, flavonoids also exhibit antibacterial activity by damaging bacterial cell membranes, inhibiting microbial enzyme activity, and disrupting nucleic acid synthesis.¹⁴ The high concentration of flavonoids in sungkai leaf extract indicates that this plant has significant potential as a source of bioactive compounds for the development of herbal medicines and natural pharmaceutical ingredients.

In addition to flavonoids, sungkai leaf extract also contains alkaloids and steroids that contribute to its biological activity. Alkaloids are known to have various pharmacological effects, such as antibacterial, antimalarial, and analgesic activities, which work by disrupting metabolism and protein synthesis in microorganisms.¹⁵ Meanwhile, plant steroids play a role in anti-inflammatory activity and can help stabilize cell membranes. The combination of these various secondary metabolites indicates that sungkai leaf extract has broad therapeutic potential and can provide synergistic biological effects.

Flavonoids are a group of polyphenolic compounds commonly found in medicinal plants and known to have strong antibacterial activity. The mechanism of action of flavonoids as antibacterial agents is mainly through their ability to damage the structure of bacterial cell membranes.¹⁶ Flavonoids can interact with proteins in the cell membrane, causing an increase in membrane permeability. As a result, important components within the cell, such as ions, proteins, and metabolites, can escape from the cell, disrupting the cell's balance and inhibiting bacterial growth.^{17,18}

Flavonoids are also able to inhibit the activity of enzymes that play a role in bacterial metabolism. These compounds can interfere with nucleic acid synthesis by inhibiting DNA replication and RNA transcription in bacteria.¹⁸ Some types of flavonoids are also known to inhibit biofilm formation, which is a protective layer formed by bacteria to defend themselves from unfavorable environments. By disrupting these important processes, flavonoids can inhibit growth and even cause bacterial cell death.¹⁹

In addition to flavonoids, alkaloids also have antibacterial potential. Alkaloids are secondary metabolites that contain nitrogen atoms and are commonly found in various medicinal plants. These compounds have antibacterial activity that works by disrupting the formation of bacterial cell wall structures. Alkaloids can inhibit the synthesis of peptidoglycan, which is the main component of bacterial cell walls. If peptidoglycan formation is disrupted, the bacterial cell wall becomes weak, making the bacterial cell susceptible to damage or lysis. Alkaloids can also interact with bacterial genetic material¹⁵. Alkaloids can bind to DNA, thereby inhibiting the replication and division of bacterial cells. Disruption of this replication process prevents bacteria from reproducing normally. In addition, some alkaloids can also inhibit the action of important enzymes involved in bacterial cell metabolism, thereby disrupting the physiological activity of bacteria and ultimately causing cell death.²⁰

Steroids are also a group of secondary metabolites that have a basic cyclic structure and are often found in plants in the form of phytosterols. These compounds have antibacterial activity that is mainly related to their ability to affect the structure of bacterial cell membranes. Steroids can interact with lipid components in cell membranes, causing changes in membrane structure and stability. These changes can increase the permeability of bacterial cell membranes.²¹ As a result of increased membrane permeability, various important components within the cell, such as ions, proteins, and nucleotides, can escape from the bacterial cell. This causes disruption of osmotic balance and metabolic processes within the cell. Damage to the cell membrane can also inhibit the transport of substances necessary for bacterial growth. Thus, steroid activity can inhibit bacterial growth and, under certain conditions, can cause bacterial cell death.²²

Clindamycin is a lincosamide antibiotic that works by inhibiting protein synthesis in bacteria. It works by binding to the 50S subunit of the bacterial ribosome, specifically to the part that plays a role in the formation of peptide bonds during protein translation. This binding inhibits the elongation of the polypeptide chain, preventing bacteria from synthesizing the proteins necessary for cell growth and reproduction. As a result, bacterial metabolism is disrupted and bacterial growth is inhibited.²³

In addition to inhibiting protein synthesis, clindamycin can also reduce the production of various bacterial virulence factors, such as toxins and enzymes that play a role in the infection process. With the reduction in the production of these virulence factors, the ability of bacteria to attack tissues and cause disease is reduced. At certain concentrations, clindamycin is bacteriostatic, inhibiting bacterial growth, but at higher concentrations or in highly sensitive bacteria, this antibiotic can be bactericidal, causing bacterial cell death. Clindamycin is effective against various Gram-positive bacteria, including *Staphylococcus aureus*.²⁴

Before being applied to the general public, toxicity testing is required. The results of the study by Muharni et al.²⁵ concluded that subchronic administration of sungkai leaf extract did not affect liver function, hematological parameters, or kidney function in albino *Rattus norvegicus* (Wistar strain) rats. The study also concluded that sungkai leaf extract is safe for use at doses below 800 mg/kg body weight²⁵.

This study has several limitations. First, the number of replications performed was relatively limited, so the variation in the data obtained was still insufficient to describe more accurate results. Second, antibacterial testing was only performed on one type of bacteria, namely *Staphylococcus aureus*, so the spectrum of antibacterial activity of sungkai leaf extract against other bacteria is still unknown. In addition, this study only used the disc diffusion method without determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), so that the antibacterial potential of the extract cannot be evaluated in greater depth.

For further research, it is recommended to test more types of bacteria, both Gram-positive and Gram-negative, and increase the number of replications to obtain more valid results. In addition, it is necessary to determine the MIC and MBC values to determine the antibacterial effectiveness more specifically. Further research can also lead to the isolation and identification of active compounds in sungkai leaf extract and testing the antibacterial mechanism in more detail.

Based on the results of the study, it was concluded that sungkai leaf extract (*Peronema canescens*) was found to contain major phytochemical compounds in the form of flavonoids, alkaloids, and steroids that have the potential to be bioactive compounds. Flavonoids were found to be the most dominant compound compared to other compounds. Antibacterial activity tests showed that sungkai leaf extract was able to inhibit the growth of *Staphylococcus aureus*, as indicated by the formation of inhibition zones at various extract concentrations. The diameter of the inhibition zone increased with increasing extract concentration, with the highest activity at a concentration of 100%. Although its activity was still lower than the positive control clindamycin, the results of this study indicate that sungkai leaf extract has potential as a natural antibacterial source that can be further developed in the field of herbal medicine.

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