

Antibacterial Potential of West Kalimantan Local Bajakah (*Spatholobus suberectus*) Ethanol Extract Against *Staphylococcus aureus* ATCC 25923 and Methicillin Resistant *Staphylococcus aureus*

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ABSTRACT

The primary factor contributing to bacterial resistance is the overutilization of antibiotics caused by *Staphylococcus aureus* ATCC25923. The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains possess a significant challenge in both clinical and community environments. Consequently, there is a need to investigate alternative antibacterial sources derived from natural ingredients and local traditional medicines. One such potential source is bajakah Jie Xue Teng (*Spatholobus suberectus*). The available data on the active component composition and antibacterial efficacy of the ethanol extract derived from bajakah (*S. suberectus*) in the West Kalimantan region is currently insufficient. The objective of this study is to evaluate the phytochemical composition and antibacterial properties of the ethanol extract derived from bajakah (*S. suberectus*), a plant species indigenous to West Kalimantan. The antimicrobial activity of the extract will be tested against two bacterial strains, namely *S. aureus* and MRSA, using in vitro methods. This study employs experimental techniques and is comprised of two distinct phases. The first phase involves conducting a phytochemical test on the ethanol extract of bajakah stem, utilizing the Thin Layer Chromatography (TLC) method. The second phase involves evaluating the antibacterial properties of the ethanol extract of bajakah stem against *S. aureus* and MRSA, employing the paper disc diffusion method. The research findings indicate that the bajakah ethanol extract derived from *S. suberectus*, a plant indigenous of West Kalimantan, possesses alkaloids, flavonoids, saponins, and steroids. The optimal antibacterial efficacy is observed at a concentration of 1,000,000 ppm, resulting in an inhibition zone diameter of 9 mm against *S. aureus* and 10 mm against MRSA.

Key words: phytochemicals; bajakah; antibacterial; *Staphylococcus aureus*; MRSA

INTRODUCTION

Antibiotic resistance is the ability of bacteria to avoid or resist the effects of antibiotics. This happens because the bacteria have undergone mutations or genetic changes so that they become resistant to antibiotics. Antibiotic resistance is a serious global problem because it can cause

infections that are difficult to treat and increase mortality (Lee Ventola, 2015; Sengupta *et al.*, 2013).

Staphylococcus aureus, a gram-positive bacterium known for its antibiotic resistance, typically lives on human skin and mucous membranes. However, when environmental conditions or the immune system are compromised, *S. aureus* can cause a variety of infections, ranging from mild to severe and life-threatening (Cheung *et al.*, 2021). One of the *S. aureus* variants that is best known for causing difficult-to-treat infections is Methicillin-Resistant *Staphylococcus aureus* (MRSA). Antibiotic-resistant

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strain infections frequently arise in epidemic waves that are started by one or a small number of successful clones. MRSA was originally associated with hospitals and other health care settings, but has now emerged as a cause of widespread community infections (Turner *et al.*, 2019). MRSA is a strain of *S. aureus* that has the ability to resist antibiotics commonly used in the treatment of bacterial infections. This includes beta-lactam antibiotics such as methicillin, penicillin, and a number of other antibiotics. In this context, antibiotic resistance means that these drugs are no longer effective in treating MRSA infections. MRSA infections can be very difficult to treat and often cause serious complications. So it poses a serious threat, especially for patients with weak immune systems, such as babies, the elderly, or individuals with chronic diseases (Depta & Niedźwiedzka-Rystwej, 2023).

Several studies show that plant extracts can inhibit the growth of *S. aureus* and MRSA with different efficacy. Study Manilal *et al.* (2020) showed that *Moringa stenopetala* and *Rosmarinus officinalis* leaf extracts have antimicrobial activity by inhibiting MRSA biofilm production. *Litsea iteodaphne* leaf extract has promising antimicrobial activity against Gram-positive and Gram-negative bacteria, including MRSA (Shashini Janesha *et al.*, 2020). Jung *et al.* (2022), conducted research on several plant extracts and fractions including gambier (*Uncaria gambir* Roxb) where the plant extract had a significant inhibitory effect on the growth of MRSA bacteria. From the results of this research, it can be concluded that plant extracts have potential as antimicrobial agents to inhibit the growth of *S. aureus* and MRSA. However, inhibitory efficacy can vary depending on the type of plant, part of the plant used, extraction method, and concentration of the extract used. Therefore, efforts to find antimicrobial agents originating from nature need to continue to be developed. Exploration of the potential of tropical plants as antimicrobial agents needs to continue to be improved, especially in the West Kalimantan region which has high plant biodiversity. One of them is bajakah Jie Xue Teng (*S. suberectus*). Bajakah is one of the plants commonly used by the

Dayak ethnic group of West Kalimantan as a medicinal ingredient.

Studies of other types of bajakah in several different regions in Indonesia have been carried out to test the antibacterial activity of bajakah extract against *S. aureus* and MRSA bacteria, including the research conducted by Latu & Suleman (2023) showed that the ethanol extract of bajakah wood had antibacterial activity against the growth of *S. aureus*. Other research conducted by Azahara *et al.* (2023) showed that bajakah tampala stem extract also had effectiveness in inhibiting the growth of *S. aureus* bacteria in vitro. From several studies, it can be concluded that bajakah extract has potential as an antibacterial against *S. aureus* and MRSA bacteria. However, research needs to be carried out to determine the potential of West Kalimantan's local bajakah extract. Remembering that the phytochemical content of plants can be different if they are in different growing environments or habitats even though they come from the same species. Research on the potential of local bajakah extract from West Kalimantan as an antimicrobial for *S. aureus* and MRSA has not been reported. Therefore, this research was conducted to evaluate the potential of bajakah extract as an antibacterial agent against *S. aureus* ATCC 25923 and Methicillin Resistant *S. aureus* (MRSA).

MATERIAL AND METHODS

Time and Place of Research

The present study was conducted in the UPT Health Laboratory of West Kalimantan Province, the Biology Laboratory of FKIP Tanjungpura University, and the Chemical Research and Biotechnology Laboratory of Tanjungpura University during the period of July to August 2022.

Research design

The study employed a Completely Randomized Design (CRD) to investigate the effects of different concentrations of ethanol extract derived from bajakah Jie Xue Teng (*S.*

suberectus). The concentrations tested included 125,000 ppm, 250,000 ppm, 500,000 ppm, and 1,000,000 ppm, each replicated four times. The experimental conditions included a positive control treatment of amoxicillin at a concentration of 30 µg and a negative control treatment of DMSO at a concentration of 10%.

Sample Preparation

Samples of bajakah wood from the local West Kalimantan Jie Xue Tang type were dried by airing and kept away from the hot sun to prevent the compound content from being damaged. After drying, the bajakah wood samples were ground into powder form, after which 230 grams of fine bajakah wood powder were obtained.

Sample Extraction

The extraction procedure employed maceration with a solvent composed of 96% ethanol. Macerate twice, each time for two 24-hour periods of immersion. In the first maceration, 3 liters of 96% ethanol were utilized, while in the second, only 2 liters were utilized. After combining the filtrate from the initial and subsequent macerations, it was evaporated at 50°C until a viscous extract of Bajakah wood was acquired. The thick extract was subsequently dried in a drying oven at 50°C for two days, yielding a final bulk of 20 grams of extract (Harborne, 1996; Yeni *et al.*, 2023).

Phytochemical Test

The phytochemical test employed the Thin Layer Chromatography (TLC) technique. The specimens were applied onto silica gel 60 F254 plates with a capillary tube. Subsequently, the TLC plate was subjected to a specific spray in order to ascertain the classification of active chemicals. The spots that were developed on TLC plate were examined using ultraviolet (UV) light at wavelengths of 254 nm and 365 nm. The spray reagents used in this study include: a) a 1% solution of FeCl₃, utilized for the detection of tannins and phenolic compounds, indicated by a green, red, or blue coloration upon spraying; b) Lieberman-Burchard reagent, used for the

detection of steroid compounds, which exhibit a blue coloration, as well as terpenoids, which display a red coloration; c) Citroborat reagent, used for the detection of flavonoid compounds, indicated by the appearance of green and yellow fluorescence; and d) Dragendorff reagent, used for the detection of alkaloids, which exhibit yellow fluorescence (Febria *et al.*, 2021; Harborne, 1996).

Preparation of Mueller Hinton Agar (MHA) Media

The MHA media was prepared by dissolving 38 grams of MHA powder in 1 L of distilled water within an Erlenmeyer flask. The mixture was then stirred and subjected to heat on a hot plate until a uniform solution was obtained. To ensure sterility, the solution was afterwards treated in an autoclave at a pressure of 1 atm and at a temperature of 121°C for a duration of 20 minutes. Subsequently, the aseptic MHA medium was transferred into a 15 mL petri dish and allowed to undergo solidification.

Preparation of Mc Farland 0.5 Solution Turbidity Standards

A McFarland turbidity standard of 0.5 was used in this study because it is equivalent to a bacterial cell suspension of 10 CFU/mL. The McFarland standard consists of 9.95 mL of 1% BaCl₂ and 1% H₂SO₄ solution, then shaken until homogeneous. McFarland standard 0.5 was used to compare *S. aureus* ATCC 25923 and MRSA bacterial suspensions.

Preparation of Bacterial Suspensions

Each bacterial colony was added to a 0.9% NaCl solution in a separate tube and the turbidity was compared with the Mc Farland 0.5 standard which is equivalent to a cell suspension of 1-2x10⁸ CFU/mL.

Testing the Inhibitory Power of Bacteria by the Disc Diffusion Method

The Kirby-Bauer disk diffusion method was employed to conduct the bacterial inhibition test. The bacterial suspension was uniformly distributed throughout the media surface using a

cotton swab. Aseptically, agar medium was inoculated with 100 µl of each bajakah extract solution, which had concentrations of 125,000 ppm, 250,000 ppm, 500,000 ppm, and 1,000,000 ppm. A concentration of 30µg/ml of Amoxicillin was used as a positive control in the experiments involving *S. aureus* ATCC 25923 and MRSA. A negative control was employed in the experiment, utilizing a 10% dimethyl sulfoxide (DMSO) solution. There were four replications for each extract and control treatment. Subsequently, the medium underwent incubation at a temperature of 27°C for a duration of 24 hours, as described by Balouiri *et al.* (2016).

Data analysis

The data obtained is presented in the form of tables and figures and then described descriptively.

RESULT AND DISCUSSION

The phytochemical test of bajakah ethanol extract was first carried out to determine the secondary metabolite content contained in the plant samples, before testing for antibacterial power.

Table 1. Phytochemical test results of Bajakah ethanol extract (*Spatholobus suberectus*).

Measurement	Results
Alkaloids (Dragondoff)	+
Flavonoids (H ₂ SO ₄)	+
Tannin (FeCl ₃)	-
Saponins (Aquades)	+
Terpenoids (libermann)	-
Steroids (Salkowski)	+
Phenolic (FeCl ₃)	-

The results of the phytochemical screening test on bajakah wood ethanol extract (Table 1) show positive results for alkaloid, flavonoid, saponin and steroid compounds. The secondary metabolite content of Bajakah stem extract has antimicrobial capabilities. According to Li *et al.* (2015) and Zhang *et al.* (2022), the main bioactive

compounds found in *S. suberectus* Dunn (SSD) are flavonoids, proanthocyanidins (PAC, condensed tannins), and other secondary metabolites including various types of alkaloids, saponins and steroids which act as antimicrobial, antioxidant, anti-inflammatory, anticancer and antiviral. This research proves that the ethanol extract of bajakah (*S. suberectus*) contains phytochemical compounds which have the potential to act as natural antimicrobials in inhibiting the growth of *S. aureus* and MRSA bacteria (Figures 1; 2).

Antibacterial Activity of Bajakah Extract

Antibacterial activity tests were carried out to determine the inhibitory potential of bajakah extract against *S. aureus* ATCC 25923 and MRSA (Figures 1; 2). Formation of a clear zone around the disc paper showed inhibitory activity against bacterial growth (Pawar *et al.*, 2020).

The average diameter of the inhibitory zone of bajakah ethanol extract against *S. aureus* ATCC 25923 at concentrations of 125,000, 250,000, 500,000 and 1,000,000 ppm was 8.5; 8.625; 7.375 and 9 mm. Meanwhile, MRSA was; 7.25; 8.5; 10.375 and 10 mm. The negative control (DMSO 10%) had no inhibitory zone. This shows that secondary metabolite chemicals in bajakah stem extract induce the inhibitory zone.

The diameter of the inhibitory zone exhibits a positive correlation with the concentration of the extract. This phenomenon can be attributed to the positive correlation between concentration and the quantity of active chemicals present in the extract. In addition, it is also influenced by the ability of the active compound to diffuse. Diffusion ability is a very important factor in determining the diameter of the inhibitory zone of plant extracts against bacteria. Diffusion is the process of moving substances from areas of high concentration to areas of low concentration. The faster a substance diffuses, the larger the diameter of the resulting inhibition zone. Factors that influence diffusion ability include the molecular size of the active compound, the physical and chemical properties of the active compound, and the physical and chemical properties of the medium used. In addition to diffusion capabilities,

The content of active compounds in plant extracts also influences the diameter of the inhibition zone against bacteria. Active compounds in plant extracts can be alkaloids, flavonoids, tannins, saponins, etc. These compounds have different antimicrobial activities against bacteria. The higher the concentration of active compounds in the plant extract, the larger the diameter of the inhibition zone produced (Naufalin *et al.*, 2021; Ningsih *et al.*, 2023; Vaou *et al.*, 2021). The alkaloid

content in bajakah extract plays an important role as an antimicrobial in the treatment of many infectious diseases because the chemical structure of heterocyclic nitrogen compounds can disrupt membrane structure by increasing the permeability of bacterial cell membranes (Cushnie *et al.*, 2014; Peng *et al.*, 2015).

Saponins are compounds found in plants and have various biological activities such as antibacterial, antifungal, antiviral, anti-

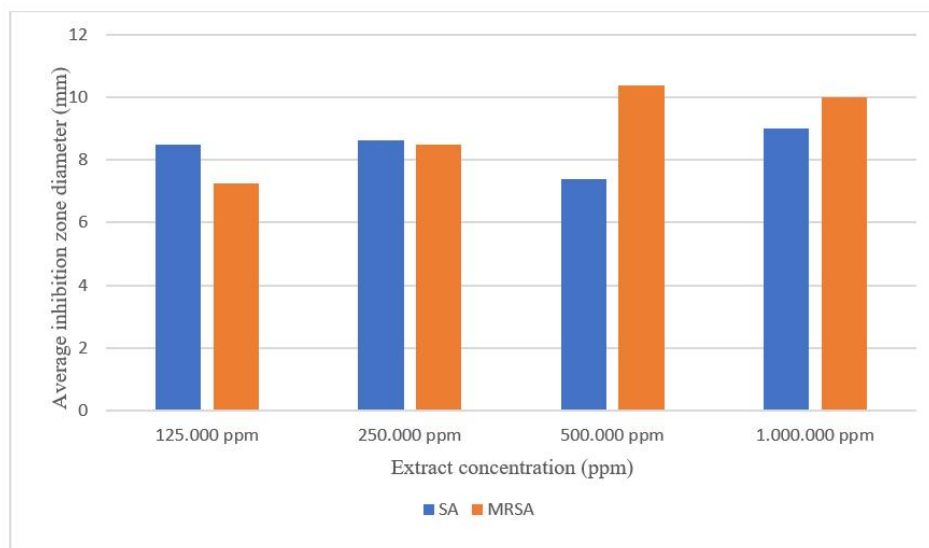


Figure 1. Average zone of inhibition of Bajakah ethanol extract (*S. suberectus*) against *S.aureus* (SA) and MRSA.

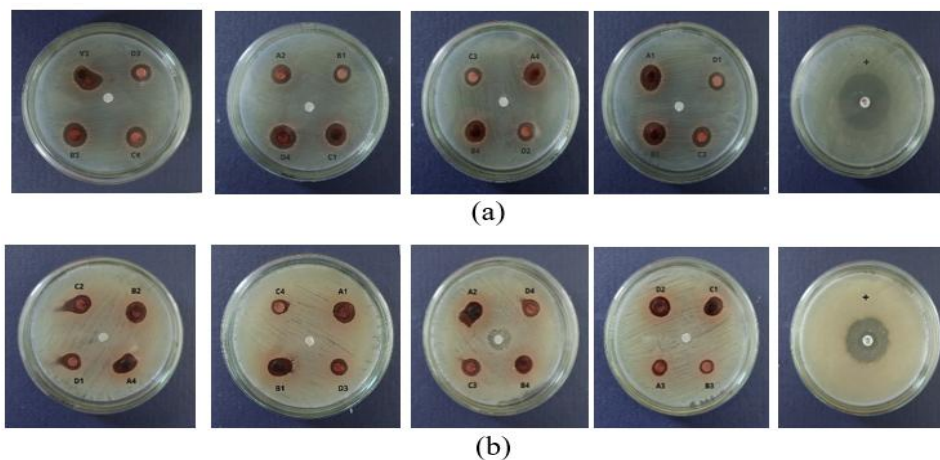


Figure 2. Inhibition test results of Bajakah ethanol extract (*S. suberectus*) against (a) *S. aureus*; (b) MRSA: A) Concentration extract 125,000 ppm; B) 250,000 ppm; C) 500,000 ppm; D)1,000,000 ppm; -) 10% DMSO negative control; +) control positive 30 mg/ml and 1,2,3,4: repeat 1,2,3,4.

inflammatory and antiulcer. The antibacterial activity of saponins is based on their chemical structure. Saponin can bind to cholesterol in cells and form a saponin-cholesterol complex which ultimately causes cell lysis. Saponins can also disrupt bacterial cell permeability by binding to the outer membrane (Khan *et al.*, 2018; Tagousop *et al.*, 2018).

The inhibition of bacterial growth by steroid chemicals can be attributed to the existence of peroxide and vinyl linking within their chemical structure. The mechanism behind the actions of steroids can be explained by their structural similarity to sterols present in the cytoplasmic membrane. This steroid has the capability to substitute chemicals within the cytoplasmic membrane, resulting in alterations to the membrane's structure. Consequently, the membrane becomes fragile and undergoes lysis (Dogan *et al.*, 2017; Vellé *et al.*, 2017).

Similar research into the anti-bacterial activity of bajakah wood has also been carried out on the growth of *S. aureus* ATCC 25923 with an inhibition zone range of 7.63 mm to 8.83 mm at an extract concentration of 10 to 30% (w/v) (Latu & Wahid Suleman, 2023). The ethanol extract of bajakah tampala (*Spatholobus littoralis* Hassk.) has the potential to be developed as an anti-biofilm candidate for *S. aureus* because it is able to inhibit *S. aureus* biofilm mid-phase 24 hours and maturation phase 48 hours (Hamzah *et al.*, 2023).

Figure 2 shows that the highest antibacterial activity of bajakah extract is at a concentration of 1,000,000 ppm at 9 mm in the medium category for *S. aureus*, while against MRSA it is 10 mm in the weak category. Measurement of antibacterial strength based on (Morales *et al.*, 2003), if no clear zone forms (-) means there is no inhibition/the extract has no potential as an antibacterial; weak/+ (6-10 mm inhibition zone) ; medium/++ (11-20 mm inhibition zone) ; strong/+++ (21 - 30 mm inhibition zone); very strong/++++ (>30 mm). The extract has potential as an antibacterial if a clear zone forms ranging from weak to very strong. The size of the inhibition zone formed as a result of extract treatment was not much different for both *S. aureus* and MRSA. This is because MRSA is

a bacteria that causes infection which comes from the *S. aureus* strain which is resistant to several groups of antimicrobials. Methicillin-resistant *S. aureus* (MRSA) is a variant of *S. aureus* bacteria that exhibits resistance to all β -lactam antibiotics, including Penicillin and Methicillin derivatives, as well as broad-spectrum beta-lactamase antimicrobials (Okwu *et al.*, 2019; Turner *et al.*, 2019).

In the positive control group (K+) containing amoxicillin at a dose of 30 mg/ml, a zone of inhibition measuring 36.5 mm was observed against *S. aureus*, while a zone of inhibition measuring 29.5 mm was observed against MRSA. Amoxicillin is a beta-lactam class of antibiotics used to treat bacterial infections, including infections caused by *S. aureus* and MRSA. Amoxicillin works by inhibiting bacterial cell wall synthesis through binding to penicillin binding protein (PBP). MRSA has resistance to beta-lactam antibiotics, including amoxicillin, due to the production of altered penicillin-binding proteins (PBPs) that are not affected by beta-lactam antibiotics. However, amoxicillin can still be used to treat infections caused by *S. aureus* that are not resistant to beta-lactam antibiotics (Yao *et al.*, 2019).

CONCLUSION

In conclusion, the research findings on the ethanol extract of bajakah Jie Xue Teng (*S. suberectus*) reveal significant potential as an antibacterial agent. This extract has been found to contain alkaloids, phenols, and flavonoids, which are known to contribute to its antibacterial effects. The highest level of antibacterial effectiveness is observed at a concentration of 1,000,000 ppm, resulting in an inhibitory zone diameter of 9 mm against *S. aureus* ATCC 25923 and 10 mm against MRSA.

One potential area for future research involves the extraction and isolation of bioactive components derived from bajakah Jie Xue Teng (*S. suberectus*) with the aim of exploring its potential as a new antibacterial agent capable of inhibiting *S. aureus* and MRSA.

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