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Antibacterial Potential of West Kalimantan Local *Bajakah* (*Spatholobus suberectus*) Ethanol Extract against *Micrococcus luteus*

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Abstract: *Bajakah Jie Xue Teng (Spatholobus suberectus) is an endemic plant from West Kalimantan which is the potential as a natural antimicrobial, but its antimicrobial ability has not been widely reported so that it can be tested on Micrococcus luteus bacteria which is the agent causing dandruff. The purpose of this study is to describe the phytochemical content of Jie Xue Teng (Spatholobus suberectus) bajakah ethanol extract and its potential to inhibit M. luteus in vitro. The phytochemical content of the extract was tested using the Thin Layer Chromatography (TLC) method. The type of research is a laboratory experiment using the Kirby Baeur disk diffusion method with swab technique with a series of extract concentrations of 1000 mg/ml, 500 mg/ml, 250 mg/ml, and 125 mg/ml, positive control (30% amoxilin), and negative control (DMSO 10%) with 4 repetitions. The phytochemical content of the ethanol extract of bajakah that was tested shows secondary metabolites of steroids, flavonoids, saponins, and alkaloids. Bajakah ethanol extract has the inhibitory potential against M. luteus for all test concentrations which fall under the moderate category. The diameter of the amoxicillin inhibition zone was still larger than the extract inhibition zone.*
Keywords: *Antibacterial; Anti-dandruff; Bajakah; Spatolobus suberectus; Micrococcus luteus.*

1. INTRODUCTION

There has been an increase in the exploration of natural materials for traditional medicine, and therefore, a lot of studies have been conducted to trace the chemical compounds contained in plants. One of West Kalimantan endemic plants with the potential for a natural medicine is *bajakah*. The local Dayak people use *bajakah* to treat diseases, such as cancer, wounds, tumors, etc. (Fitriani et al., 2020). In addition, it is also used to treat fever, headaches, digestive disorders, microbial infections, high blood pressure, and neurological conditions (Ravipati et al., 2014). Zhang (2015) also reported that *bajakah* has bioactivity as a medicine for rheumatism, diabetes, asthma, stroke and cancer.

Bajakah comes in several types, such as lamei, kalalawit, and tampala (Amiani et al., 2022). *Bajakah Jie Xue Teng* (*Spatholobus suberectus*) is commonly found in the forests of West Kalimantan, but has not been optimally utilized. *Bajakah tampala* contains bioactivity as an antioxidant and compounds of phenolics, flavonoids, tannins and alkaloids which have anti-breast cancer potential (Abdulrahman et al., 2021; Maulidie et al., 2019). According to Zhang (2015), plants of the *Uncaria* genus have more than 200 secondary metabolites with the main compounds containing alkaloids, terpenoids, and flavonoids. Similar research also reported that the phytochemical content of the stem and bark extracts of the *Bajakah* contains various secondary metabolites with the main compound groups containing terpenoids, flavonoids, and alkaloids (Flores-Sanchez & Ramos-Valdivia, 2017; Iskandar & Warsidah, 2020; Maulina et al.,

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2019). Park et al., (2017) reported that *bajakah* extract can inhibit the growth of *S. aureus* by isolating seven flavonoid compounds from Spatholobus sp. through high performance liquid chromatography. Using column chromatography instruments, Tang et al., (2012) found that the *bajakah* ethanol extract contains 17 chemical components. There are 20 compounds isolated from the hanging roots of *Spatholobus sp.*, 2 flavonoid compounds, namely 7-hydroxy-6-methoxy flavano formononetin which can inhibit *S. aureus* (Cho et al., 2017). Kurniawan, (2019) also reported that the stem of the *bajakah tampala* (*Spatholobus littoralis Hassk*.) showed antibacterial activity by inhibiting the growth of *Staphylococcus aureus* ATCC 26923 at a concentration of 100 percent.

Information on the *bajakah* potential inhibition against several types of bacteria has been reported, but research on the content of metabolites of the *Jie Xue Teng (Spatholobus suberectus)* stem from West Kalimantan and its potential inhibition against Micrococcus luteus have not been found. The *micrococcus luteus* bacterium is one of the normal microflora of human skin which accounts for 20-80% isolated from human scalp, feet and arms (Kloos & Musslewhite, 1975; Sims & Sommers, 1986). Changes in the physiological conditions of the host's body can affect on the number of microflora living on the skin, one of which can increase secretion of sebum. Sebum contributes most of the lipid components on the skin surface to maintain skin hydration so that it stays moist and not dry (Hoover et al., 2022). Secretion of sebum will increase throughout a person's teenage years, especially at the age of 15 to 35 years which is the peak (Jacobsen, 1985) can be a source of nutrition for bacterial growth. In addition, the moisture maintained by sebum becomes a desirable environment for the growth of various species of bacteria, one of which is *M. luteus* (Byrd et al., 2018; Xu et al., 2016). Under these conditions, the *M. luteus* bacteria can thrive on the scalp and form colonization which causes dandruff indicated by itchy and flaky scalp (Grimshaw et al., 2019).

To overcome the dandruff problem, people usually use anti-dandruff shampoo that contains active ingredients, such as ketoconazole, salicylic acid, zinc pyrithione, coal tar, dipyrithion, selenium sulfide, pyroctone olamine, and hydrocortisone (Sweetman, 2009). However, the active ingredients in anti-dandruff shampoo have side effects, such as causing hair loss and pain to the scalp. Hair loss is caused by the keratolytic effect of shampoo and pain on the scalp caused by cuticle cells that are lifted from damaged human skin cells (Rajput, 2017). These cells will be tied up together so that the hair becomes tangled. These side effects have triggered people to switch to natural-based products to treat dandruff instead of using synthetic chemicals which are harmful to the skin. Therefore, this research was conducted to examine the content of secondary metabolites of the Jie Xue Teng *bajakah* stem and determine their potential to inhibit the growth of *M. luteus* bacteria that infect the scalp.

2. RESEARCH METHODOLOGY

Time and Location of Research

This research was conducted at the Technical Implementing Unit of Health Laboratory of West Kalimantan Province, the Biology Laboratory of FKIP Tanjungpura University, and the Chemical Research and Biotechnology Laboratory of Tanjungpura University from April to July 2022.

Tools and materials

The tools used in this study were autoclaves, beakers, measuring cups, ovens, pipettes, petri dishes, incubators, Erlenmeyer flasks, Bunsen burner, refrigerator, stopwatch, loop needles, test tubes, glass stir bars, test tube racks, plugs, analytical scales, stationery, camera, hot plate, micropipette, funnel, syringe, bulb, and scissors.
The materials used in this study were *bajakah* (Spatholobus suberectus), *M. luteus* bacteria, Mueller Hinton Agar (MHA), 96% ethanol, sterile distilled water, aluminum foil, plastic wrapping, antibiotics (Amoxicillin 30µg/disc), physiological NaCl 0.9%, sterile cotton buds, plugs, straw paper, plastic puppets, tissue, 70% alcohol, matches, label paper, Whatman filter paper number 1 measuring 125 mm, and disc paper measuring 6 mm.

Research Design

This research used a Completely Randomized Design (CRD) with 4 series of

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concentrations of the the Jie Xue Teng (*Spatholobus suberectus*) ethanol extract, namely 125,000 ppm, 250,000 ppm, 500,000 ppm, and 1,000,000 ppm with 4 replications. Positive and negative control treatments were conducted using amoxicillin 30 µg and DMSO 10% respectively.

Sample Preparation

The local Jie Xue Tang type of Bajakah wood from West Kalimantan was dried and kept from the sun in order to preserve the compound content from being damaged. After drying, the samples of the *Bajakah* wood were crushed into powder. As much as 230 grams of fine powdered *Bajakah* wood was obtained.

Sample Extraction

The extraction process used the maceration method with 96% ethanol solvent. The maceration was conducted twice with immersion of 2x24 hours for each maceration. The first maceration used 3 liters of 96% ethanol and the second maceration used 2 liters. The filtrate resulting from the first and second maceration was mixed and then evaporated at a temperature of 50ºC to obtain a thick extract of *Bajakah* wood. The viscous extract was then dried for 2 days in an oven at a temperature of 50ºC and a final mass of 20 grams of the extract was obtained.

Preparation of Mueller Hinton Agar (MHA) Media

MHA media was prepared by adding 38 grams of MHA powder into 1 liter of distilled water in an erlenmeyer flask, and it was stirred, then heated on a hotplate to make the solution homogeneous, sterilized by autoclaving at 1 atm air pressure and 121°C for 20 minutes. Sterile MHA media was then poured into a 15 mL petri dish and allowed to solidify.

Preparation of Standard Turbidity McFarland Solution 0.5

Mcfarland turbidity standard of 0.5 was used in this research because it is equivalent to 10 (CFU)/mL bacterial cell suspension. McFarland standards consist of 9.95 mL of 1% BaCl2 and 1% H2SO4 solution. Then it was shaken until it bacame homogeneous. The McFarland standards of 0.5 were used to compare suspensions of *M. luteus* bacteria.

Preparation of Bacterial Suspensions

Bacterial colonies were added to each 0.9% NaCl solution in separate tubes and compared for turbidity with McFarland standards 0.5 which is equivalent to 1-2x108 CFU/mL cell suspension.

Testing the Inhibitory Power of Bacteria with Disc Diffusion Method

Bacterial inhibition test was conducted using the Kirby-Bauer disc diffusion method. The bacterial suspension is smeared evenly on the surface of the media using a cotton swab. Disc paper that had been dripped with 100µl of each *Bajakah* extract solution with a concentration of 1000 mg/ml, 500 mg/ml, 250 mg/ml, and 125 mg/ml respectively was placed aseptically on the agar medium. Amoxicillin 30µg/ml was used as a positive control for *M. luteus*. As for the negative control 10% DMSO was used. Each extract and control treatment had 4 replications. Then, the media was incubated at 27°C for 1x24 hours.

Data Analysis

The results of measuring the inhibition zone diameter of *M. luteus* bacteria were then analyzed using the RAL model Analysis of Variant (ANOVA). Then, a post-hoc analysis conducted to see the differences between treatments and to look for extract concentrations that had the same inhibitory effect as amoxicillin. Data analysis was conducted using the SPSS application version 25.

3. RESULTS AND DISCUSSION

Before testing the inhibitory power of the Bajakah ethanol extract, the extract was tested for its phytochemical content in order to identify the group of metabolites in the extract of *Bajakah* sawdust. The phytochemical content test can provide an overview of the compound content in Bajakah sawdust so that the effect of using the extract of Bajakah sawdust as an inhibitor for M. luteus bacteria could be identified. The phytochemical test data for Bajakah wood extract is shown in Table 1.

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Table 1. Test for Phytochemical Content of *Bajakah* Ethanol Extract

Measurement	Result
Alkaloid (Dragondoff)	+
Flavonoid (H_2SO_4)	+
Tanin ($FeCl_3$)	-
Saponin (Aquades)	+
Terpenoid (libermann)	-
Steroid (Salkowski)	+
Fenol ($FeCl_3$)	-

The tested Bajakah ethanol extract contains secondary metabolites of steroids, saponins, flavonoids, and alkaloids (Table 1). The existence of this class of compounds is closely related to the antibacterial potential of the extract which is indicated by the presence of an inhibition zone (Figure 1). The size of the inhibition zone for all extract concentration treatments can be seen in Table 2.

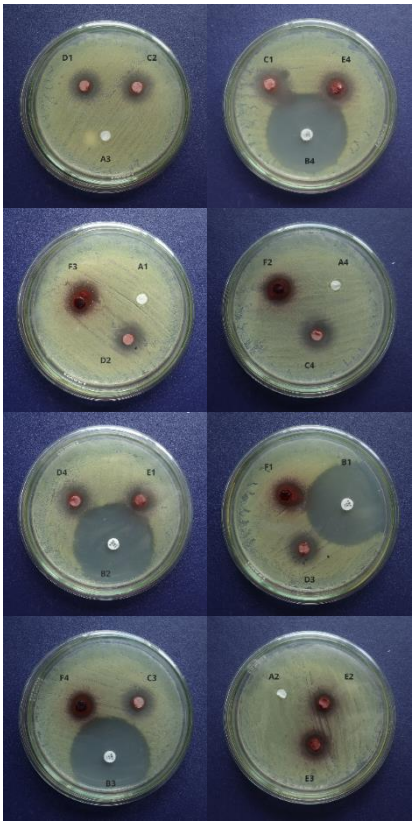

Figure 1. Inhibition zones for the growth of M. luteus formed from each extract treatment: A) DMSO 10%; B) Amoxicillin 30 µg; C) Extract 125,000 ppm; D) Extract 250,000 ppm; E) 500,000 ppm; F) 1,000,000 ppm.

Tabel 2. Results of measuring the inhibition zone diameter of the Bajakah extract for the growth of *M. luteus*

Treatment	Inhibition Zone Diameter	Category
DMSO 10%	0,00	-
Amoxicilin 30µg	36.50 ± 1.08	Very Strong
Ekstract 125.000 ppm	8.50 ± 1.83	Moderate
Ekstract 250.000 ppm	8.63 ± 1.44	Moderate
Ekstract 500.000 ppm	7.34 ± 1.60	Moderate
Ekstract 1.000.000 ppm	9.00 ± 1.48	Moderate

This research shows that the content of phytochemical compounds in the *Bajakah* (*Spatholobus suberectus*) ethanol extract has the potential for a natural antimicrobial in inhibiting the growth of *M. luteus* bacteria (Table 1). Based on the category of the strength of inhibition according to Davis & Stout (1971) in Fachriyah et al., (2020) the average diameter of the growth inhibition zone of *M. luteus* by the *Bajakah* ethanol extract at concentrations of 125,000 ppm, 250,000 ppm, 500,000 ppm, and 1,000,000 ppm respectively were 8.50 ± 1.83; 8.63±1.44; 7.34±1.60; and 9.00±1.48. These results explain that an increase in the extract concentration will be followed by an increase in the compound content in the solution.

The increase in extract concentration has caused the viscosity of the extract solution to increase as well. Viscosity with regard to the level of viscosity of the solution can affect the spreadability of the extract on the growth media. According to Siska et al., (2019), when the viscosity of the extract solution increases, the spreadability of the extract on the media decreases. In contrast, decreasing the viscosity of the solution will increase the spreadability of the extract on the media. Therefore, the extract solution with a lower concentration will spread more easily on the media so that it inhibits bacterial growth more rapidly. However, in addition to affecting the spreadability of the

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solution, the viscosity of the solution also affects the total compound content in the extract solution. The high viscosity of the solution is directly proportional to the total compound content in the extract solution. Therefore, even at high concentrations, the extract solution is slower to spread on the media due to its high viscosity but the content of the compounds in the extract solution is also higher. The compounds in the extract solution will accumulate in the growth media so that they can inhibit microbial growth more effectively (Madigan, 2012).

The alkaloid group of compounds works by inhibiting the synthesis of nucleic acids and proteins which cause damage to the DNA/RNA of bacterial cells so as to prevent the expression of genes that affect the growth and reproduction of these bacterial cells (Othman et al., 2019). Alkaloids also damage bacterial cell membranes by breaking down the membrane layer which includes phospholipid bilayers and proteins, causing the release of a large number of molecules and disrupting the defense and transport functions of cells (Mabhiza et al., 2016). The flavonoid compound group is polar in nature because it has a hydroxyl group in its chemical structure. Flavonoids can be a good growth inhibitor in gram-positive bacteria. In this research, *M. luteus* is a group of gram-positive bacteria. The cell wall of gram-positive bacteria contains polysaccharides which are water-soluble polymers, making it easy for flavonoids to penetrate the bacterial cell wall and damage its components (Echeverria et al., 2017; Osonga et al., 2019). Phenolic compounds also have hydroxyl groups on their aromatic rings. Substitution of hydroxyl groups that have lipophilic properties can damage lipid components and increase the permeability of the cytoplasmic membrane (Borges et al., 2013; Liu et al., 2020). The mechanism of bacterial growth inhibition by steroid compounds is due to the presence of peroxide and vinyl bonds in their structure (Vida et al., 2012). The steroid effect can be explained by the fact that there is a resemblance to the sterols present in the cytoplasmic membrane. These steroids can replace substances in the cytoplasmic membrane which cause changes in the structure of the membrane, causing the membrane to become brittle and disintegrate [lysis] (Dogan et al., 2017; Glauert et al., 1962). Saponins diffuse through the cell wall and then bind to the cytoplasmic membrane which contains lipid components so as to increase cell permeability and cause leakage of intracellular components. Saponins are referred to as antibacterial agents with a working mechanism that damages the permeability of the cytoplasmic membrane (Cavalieri et al., 2005; Nuria et al., 2009).

The negative control used was 10% DMSO because it can dissolve polar and nonpolar compounds with a low level of toxicity (Galvao et al., 2014; Suwandi, 2009). The positive control used was Amoxicillin 30µg/ml. Amoxicillin belongs to a group of antibiotics with a broad spectrum that is effective for inhibiting the growth of gram-positive and gram-negative bacteria (Kaur et al., 2011). Amoxicillin inhibits bacterial growth through inhibition of mucopeptides which are required in bacterial cell wall synthesis (Nagaralli et al., 2002). The inhibition occurs in the process of cross-linking in bacterial cell wall synthesis (transpeptidation) in the activation of autolytic enzymes which causes lysis of the bacterial cell wall so that it destroys the bacterial cell (Akhavan et al., 2022; Bernatova et al., 2013).

Further tests using Duncan's multiple range test to find the best concentration showed no significant difference between the four extract concentrations. On the contrary, the best treatment was amoxicillin as a positive control which was significantly different from the four extract solutions. The extract used in this research was a mixed extract so that the content of specific active compounds in the extract had not been obtained. Nevertheless, the extract of *Bajakah* has the potential as an antioxidant and antimicrobial, especially in inhibiting the growth of bacteria.

4. CONCLUSION

Based on the research that we conducted, it proves that the ethanol extract of the Jie Xue Teng (*S. suberectus*) type of Bajakah wood contains secondary metabolites of flavonoids, saponins, alkaloids, and steroids. The compound contents in the extract have the potential to inhibit *M. luteus* bacteria in the four series of concentrations, which

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fall under the moderate category. It is necessary to conduct further tests in the future in order to find out the right dose to inhibit stronger bacteria. In this case, the ethanol extract of the Jie Xue Teng type of *bajakah* wood can be used as an alternative antimicrobial medicine.

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