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Research Article

The Potential of Bajakah Endophytic Fungus (*Spatholobus littoralis Hassk*) Against Staphylococcus aureus Bacteria

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Abstract: A plant that has great potential as a source of traditional medicine is Spatholobus littoralis Hassk, or better known as bajakah. The research aims to analyze the Potential of Bajakah Endophytic Fungi (Spatholobus littoralis Hassk) Against Staphylococcus aureus Bacteria. This research was conducted at the Pharmaceutical Microbiology Laboratory, Faculty of Health, Sari Mulia University, Banjarmasin. The study used endophytic fungi on Bajakah wood (Spatholobus littoralis Hassk). The research design used was a True Experimental design with the aim of identifying the antibacterial activity of Bajakah endophytic fungi (Spatholobus littoralis Hassk) and testing the inhibitory power of Bajakah endophytic fungi (Spatholobus littoralis Hassk) against Staphylococcus aureus bacteria. Three isolates of endophytic fungi from Bajakah stems (Spatholobus littoralis Hassk) showed antibacterial activity against Staphylococcus aureus bacteria with varying levels of potency. Isolate (JEB 1) which is estimated to originate from Aspergillus sp showed very strong antibacterial activity with an inhibition zone diameter of 27.18 ± 0.725 mm. Isolate (JEB 1) has a Minimum Inhibitory Concentration (MIC) value at a concentration of 5 x 106 ppm and the best Minimum Bactericidal Concentration (MBC) at a concentration of 5 x 106 ppm. These findings indicate that endophytic fungi, especially from Aspergillus sp, have potential as natural antibacterial agents that can be further developed as candidates for new antibacterial materials based on local natural resources.

Keywords: Bacteria; Bajakah; Endophytic Fungi; Plant; Staphylococcus Aureus.

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1. Introduction

Infectious diseases caused by bacteria remain a serious global health problem, particularly infections caused by *Staphylococcus aureus*. S. aureus is a Gram-positive bacterium that can cause a variety of diseases, from skin infections and pneumonia to sepsis and endocarditis. The biggest challenge in managing these infections is the emergence of antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA), which is difficult to treat due to its resistance to various conventional antibiotics. The WHO (2024) reports that approximately two million MRSA infections occur annually worldwide, with the majority of cases found in developing countries.

In Indonesia, the prevalence of antibiotic-resistant S. aureus infections is also quite high. According to a report by the Indonesian Ministry of Health (2020), approximately 38% of S. aureus infections in Indonesian hospitals are methicillin-resistant strains. This situation indicates that the use of synthetic antibiotics is increasingly ineffective, necessitating efforts to develop safer, more effective, and environmentally friendly alternative treatments. One promising alternative is the use of traditional medicinal plants and the microorganisms that live in symbiosis with these plants.

A plant with great potential as a source of traditional medicine is *Spatholobus littoralis Hassk*, better known as bajakah. This plant is commonly found in the forests of Kalimantan and has long been used by the Dayak people to treat various ailments such as diarrhea, wounds, cancer, and diabetes. Research shows that bajakah contains bioactive compounds such as flavonoids, saponins, and tannins, which have potential antibacterial, anti-

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inflammatory, and anticancer properties (Abdulrahman et al., 2022). Inland communities in Halong District use a decoction of bajakah tampala stems as a traditional remedy for diarrhea, indicating the plant's natural antibacterial activity.

Scientific research supports this traditional use. Rohmania (2021) reported that ethanol extract of bajakah stems exhibited significant antibacterial activity against *Staphylococcus aureus* and Escherichia coli. These findings were confirmed by Mochtar et al. (2022) reported that ethanol extract of bajakah tampala stems had an inhibitory effect of 83.21% ± 0.01% against S. aureus, even higher than ciprofloxacin as a positive control. Furthermore, bajakah extract also exhibited antifungal activity against Candida albicans (Mochtar et al., 2022).

Besides its antibacterial activity, bajakah tampala also possesses anti-inflammatory and antioxidant effects. Nastiti & Nugraha (2022) found that bajakah extract can reduce inflammation by inhibiting the activity of the peroxidase enzyme and reducing inflammatory cytokines such as IL-1RA, iNOS, and COX-2. Another study by Iskandar et al. (2022) showed that the n-hexane and ethyl acetate fractions of bajakah have cytotoxic activity against breast cancer cells with IC_{50} values of 20.0 μg/mL and 7.4 μg/mL, respectively, and exhibit strong antioxidant activity with an IC_{50} value of 198.76 ppm.

Overexploitation of bajakah plants in nature can threaten their sustainability. Therefore, a sustainable biotechnological approach is needed to obtain bioactive compounds without damaging plant populations in their natural habitat. One potential approach is through the exploration of endophytic fungi that live symbiotically within plant tissues without causing detrimental effects to their hosts. Endophytic fungi are known to produce secondary metabolite compounds similar to those of their host plants. Research by Oktavira et al. (2024) reported that endophytic fungi from bajakah stems have strong antibacterial activity against Escherichia coli with an inhibition zone of 24.42 ± 5.28 mm.

Exploring the potential of endophytic fungi derived from Bajakah (*Spatholobus littoralis Hassk*) stems as antibacterial agents against *Staphylococcus aureus* is crucial. This research is expected to broaden knowledge about the role of endophytic fungi as an alternative source of natural antibacterial compounds with the potential to be developed into new, safer, more effective, and environmentally friendly drugs. The research aims to analyze the potential of Bajakah (*Spatholobus littoralis Hassk*) endophytic fungi against *Staphylococcus aureus* bacteria.

2. Literature Review

The Bajakah Tampala plant (*Spatholobus littoralis Hassk*) is a climbing plant on Karl's wood tree from the Phaseolea family with a height of approximately 50 meters, has 29 species that grow in tropical forests of Indonesia (Kurnianto et al., 2020). Bajakah was first discovered in 1842 by botanist Justus Karl Haskar and is widespread in Asia. There are 29 species living in the forests of Southeast Asia (Maulidie et al., 2019). Bajakah (*Spatholobus littoralis Hassk*) belongs to the Fabaceae family, which includes closed seed plants and consists of 500 genera and 11,500 species. A characteristic of the Fabaceae family is that the fruit emerges from carpels with or without pseudosepta. When the seeds are ripe, a stomach cavity forms, the seeds are released, and the fruit divides into several parts according to the pseudoseptum. This plant has a very large and strong stem. The roots of this plant grow very long, up to 5 meters (Istiqomah et al., 2021). The bajakah tree can grow up to 50 meters tall. Its leaves are pointed and colored yellow, white, and brown. The plant also bears small flowers in various colors, including purple, white, and pink. The roots of this plant can grow long and drooping, reaching over 5 meters. When the stem is cut, it releases a liquid that is safe for consumption (Prasetyorini et al., 2022).

Fitriani et al. (2020) found that the bark and stem of the bajakah tree also contain phenolic compounds, alkaloids, and terpenoids. This is supported by research by Istiqomah et al. in 2021, which found that the bajakah tree stem tested positive for flavonoids, saponins, tannins, and phenols. According to research by Maulidie et al., ethanol extract of bajakah has been shown to be effective in inhibiting the growth of the highly toxic Escherichia coli bacteria, which has a diameter of 20.32 mm, and has the potential to be used as a raw material for medicines (Maulidie et al., 2022).

Endophytes are microorganisms that live within plant tissue and can colonize without harming or harming their host plants. Every higher plant can harbor several microbes, one of which is endophytes, which are capable of producing bioactive compounds or secondary metabolites due to genetic transfer from their host plant to the endophyte (Hidahyati, 2010). Endophytes are divided into two types: fungal endophytes and bacterial endophytes.

Endophytic fungi are fungi that live within plant tissue without causing harmful disease symptoms to their host plants. Endophytic fungi can be found in various plant parts such as leaves, stems, roots, and other tissues. The presence of these fungi often provides benefits to plants, such as increased resistance to pathogens, environmental stress, or herbicides. Endophytic fungi can help plants survive unfavorable conditions, such as nutrient-poor soils or dry environments. Endophytic fungi also play a role in improving plant access to nutrients through a symbiotic relationship, where the fungi can help plants better absorb nutrients, such as phosphorus or nitrogen. Some endophytic fungi are also capable of producing bioactive compounds that function to fight pathogens or other organisms that compete with plants. Some well-known types of endophytic fungi include the genera Fusarium, Penicillium, and Cladosporium (Jaber & Enkerli, 2017).

The use of antibiotics is a solution to treat various infectious diseases. However, the continuous use of synthetic antibiotics not only kills the pathogens themselves but also accelerates the development of resistance, and the raw materials for synthetic antibiotics are still imported. Therefore, alternative, safe, and naturally occurring antimicrobial compounds are needed (Wathan, Imaningsih, & Rizki, 2021). A widely explored source of new bioactive compounds is endophytic fungi. Endophytes are bacterial or fungal microorganisms that live intercellularly in healthy plant tissue (Zheng, Li, Zhang, & Zhao, 2021). Penicillium sp. is an endophyte found in certain plants, such as medicinal plants, and has also produced compounds such as taxol, podophyllotoxin, and camptothecin, antibacterials similar to penicillin, which can inhibit the growth of gram-positive bacteria.

Staphylococcus aureus is a spherical, Gram-positive bacterium with a diameter of 0.7-1.2 μm, which grows in irregular clusters like grapes, does not form spores, is facultatively anaerobic, and is non-motile. The optimum temperature for its growth is 37 0C, but at room temperature (20 0C – 25 0C) it will form pigment. The color of the pigment formed ranges from gray to golden yellow with colonies that are round, smooth, raised, and shiny. More than 90% of clinical isolates show the morphology of *Staphylococcus aureus* with a polysaccharide capsule or thin membrane that plays a role in bacterial virulence. *Staphylococcus aureus* infection is one of the causes of increasing numbers of diseases and deaths. In the nose and human skin, there are bacteria that colonize so that they can cause several diseases such as skin infections, endocarditis, bacteremia, pneumonia, meningitis, osteomyelitis, sepsis and toxic shock syndrome. One of the challenges in treating infections by *Staphylococcus aureus* is the presence of Vancomycin-Resistant *Staphylococcus aureus* (VRSA), namely the resistance of *Staphylococcus aureus* to antibiotics. In addition, the emergence of new strains of *Staphylococcus aureus* also adds to public health problems. Therefore, new strategies are needed to avoid the spread of resistance (Wikananda et al., 2019).

3. Materials and Method

This research was conducted in the Pharmaceutical Microbiology Laboratory, Faculty of Health, Sari Mulia University, Banjarmasin. The study used endophytic fungi from Bajakah wood (Spatholobus littoralis Hassk). The study used a True Experimental design with the aim of identifying the antibacterial activity of Bajakah endophytic fungi (Spatholobus littoralis Hassk) and testing their inhibitory efficacy against Staphylococcus aureus.

The research procedure began with the collection of Bajakah wood samples (Spatholobus littoralis Hassk) from Mamigang Village, Halong District. Bajakah stems were thoroughly washed using running water, then sterilized by immersion in 70% alcohol for 5 minutes and 1% NaOCl solution for 5 minutes to remove contaminants. Then, they were rinsed with sterile distilled water and dried. Next, stem cuttings were aseptically planted on PDA media for endophytic fungal isolation. Tools such as petri dishes, loops, and tweezers were sterilized in an oven at 180°C for 2 hours, while glassware and materials were sterilized in an autoclave at 121°C for 15 minutes. The grown fungal isolates were incubated at 25°C for 10 days, observed macroscopically and microscopically to identify the morphology and structure of hyphae, conidia, and conidiospores, with the option of molecular identification using PCR-ITS if necessary.

Antibacterial activity was tested against Staphylococcus aureus. A 0.5 McFarland standard was prepared by mixing 1% $BaCl_2$ and 1% H_2SO_4 solutions, then its absorbance was measured (0.08–0.10). The bacterial suspension was adjusted to this standard before use. PDA and NA media were prepared with their respective compositions and sterilized at 121°C for 15 minutes. Antibacterial tests were conducted using the disc diffusion (Kirby-Bauer) and dilution methods. In the disc diffusion method, endophytic fungal supernatant was dropped onto a paper disc placed on NA media containing S. aureus, then incubated for 24 hours at 37°C to measure the inhibition zone. In the dilution method, variations in supernatant concentration were used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) based on turbidity and colony growth after incubation.

4. Results and Discussion

4.1. Determination Results

The results of the determination confirm that the plant used in this study is indeed the Bajakah tampala plant, of the species Spatholobus littoralis Hassk. Morphologically, Bajakah tampala has a woody stem, small to medium in diameter, and grows climbing other trees, reaching tens of meters in length. The stem is reddish-brown with a slightly rough surface. The leaves are compound, elliptical to oblong with flat edges and pointed tips, dark green on top and light green on the underside. The flowers are reddish-purple with a distinctive bean-like shape, while the fruit is a flat pod that turns dark brown when ripe.

4.2. Sampling

Sampling of Bajakah (Spatholobus littoralis Hassk) stems in this study was conducted on March 8, 2025, in Mamigang Village, Halong District, Balangan Regency, South Kalimantan Province. This area was chosen because it is one of the natural habitats of the bajakah plant, so it is expected that the bioactive compounds in the plant will be at optimal levels. The sample used was a single bajakah tampala stem. The outer part of the stem was taken and sampled, as various previous studies have shown that this part of the stem contains important secondary metabolites such as flavonoids, phenols, tannins, and saponins, which have potential antioxidant and antibacterial properties (Sari et al., 2023). Previous research conducted by Saparudil Latu et al, (2023) provided antibacterial activity against the growth of Staphylococcus aureus bacteria using the paper dizk diffusion method using concentrations of 10% w/v, 20% w/v and 30% w/v. The results showed that Bajakah Wood Extract has antibacterial activity at a concentration of 10% obtained an average inhibition zone of 7.63 ± 0.15 mm, a concentration of 20% obtained an average inhibition zone of 8.26 ± 0.28 mm and a concentration of 30% obtained an average inhibition zone of 8.83 ± 0.40 mm. The conclusion of this study is that a concentration of 10% with an inhibition zone of 7.63 ± 0.15 mm can inhibit the growth of Staphylococcus aureus. The results of the observation indicate that the Endophytic fungus of Bajakah stems (Spatholobus littoralis Hassk) can grow through tissue. This statement is supported by Monika et al., (2020). Endophytic fungi within plant tissue can produce bioactive compounds that act as triggers for the host plant to produce secondary metabolites, or active compounds that mimic those of their host. Endophytic fungi can produce compounds that bacteria biosynthesize.

4.3. Sample Sterilization

Sterilization is a crucial process that must be completed before conducting research involving microorganisms. Sterilization is performed on all tools and materials used in experiments, including laboratory equipment and fungal growth media. Sterilization kills all pathogenic microbes, preventing them from multiplying and contaminating the test material (Hanifah et al. 2021). The sterilization of tools and microbial growth media in this study involved physical sterilization using heat from an autoclave. The heat comes from water vapor, thus referred to as wet sterilization. Condensation creates dew, which creates sufficient moisture to kill germs, thus rendering the material sterile (Syah, 2016).

Sample sterilization is also crucial in this study to ensure that the endophytic fungi grown are truly endophytic fungi and not pathogenic microbes. The sterilization process for Bajakah Tampala (Spatholobus littoralis Hassk.) stems began by cutting the bark into small pieces. The pieces were then soaked in a 70% alcohol solution for two minutes to kill or reduce the number of contaminating microorganisms, including bacteria and viruses (Megawati & Fatmala, 2017). Next, the pieces of wood were soaked in a 5% NaOCl solution for two minutes to sterilize the surface. This step is crucial to inhibit the growth of non-target microorganisms, such as external fungi, so that only bacteria from the stem surface can thrive (Rahayu, 2018). Afterward, two rinses were performed with sterile distilled water, each for two minutes, to ensure that the target microorganisms remained viable and were not completely eliminated (Rahayu, 2018).

4.4. Growth of Bajakah Stem Endophytic Fungus (Spatholobus littoralis Hassk)

This study used samples of Bajakah stem plants (Spatholobus littoralis Hassk), which have antibacterial potential and are widely used by local communities as a remedy for itchy skin. Itchy skin is a disease caused by the bacteria Staphylococcus aureus. This study used samples of Bajakah (Spatholobus littoralis Hassk) stems taken directly from Halong District. Endophytic fungi were then grown on them, as they contain numerous secondary metabolites. Three types of fungi were found growing on the Bajakah (Spatholobus littoralis Hassk) stems. The presence of endophytic fungi can be linked to the habitat conditions of the host plant, which can lead to differences in the types of fungi that grow. Several factors influence the growth of endophytic fungi, such as environmental factors around the host plant, soil pH, and the type of tissue in the host plant (Manurung et al., 2022).

4.5. Purification of Endophytic Fungi from Bajakah Tampala (Spatholobus littoralis Hassk) Stem Endophytes

The initial growth of microorganisms went through a purification stage by isolating each fungal colony that had a different color on the surface of the Bajakah stem. The purification process was carried out by cultivating fungi that had previously grown on PDA media with similar characteristics and grew on the Bajakah stem. These endophytic fungi were then re-cultivated in a new, previously prepared growth medium.

The isolation results yielded three types of endophytic fungi based on differences in colony color: black fungi (JEB1), white fungi (JEB2), and gray fungi (JEB3). This finding aligns with the research objective, which was to identify the diversity of endophytic fungi found in the bark of Bajakah Tampala (Spatholobus littoralis Hassk). Each pure isolate was then subcultured onto new media to create duplicates, both as working cultures in the research and as stock cultures.

4.6. Morphology of Bajakah Stem Endophytic Fungi

The morphology of endophytic fungi was determined both macroscopically and microscopically to identify the characteristics of each isolate. Macroscopic observations included colony color, surface texture, colony shape, and the underside of colonies grown on Potato Dextrose Agar (PDA) media. Microscopic observations, conducted using an optical microscope at 40× magnification, included hyphal structure (septate or not), hyphal color, conidia shape and arrangement, and spore morphology. This method serves as the basis for fungal identification because their vegetative and reproductive structures show specific differences between species (Ismail et al., 2018).

Observations using an Optilab microscope showed that the isolate (JEB 1) had a hyphal structure resembling fine roots, with spores attached to the tips of the hyphae. This morphology is similar to the fungus Aspergillus sp., which is characterized by the presence of upright conidiophores ending in vesicles, structures where phyllids form, producing round, brown conidia. Aspergillus sp. is known to produce bioactive compounds with antibacterial properties (Sumilat & Lintang, 2021). Observations of isolate (JEB 2) using an optilab microscope revealed white, circular, and cotton-like colonies, suggesting a similarity to Mycelia sterilia, whose mycelial structure is white and cotton-like. Meanwhile, observations of isolate (JEB 3) using an optilab microscope revealed light gray, elongated, thread-like hyphae with branching appearance. The gray color indicates that this fungus belongs to the group of hyaline fungi that lack dark pigmentation. The observed spores appeared round and oval, scattered around the hyphae. Microscopic staining clarified the internal structure of the hyphae and spores, such as the cell wall and septum. Isolate (JEB 3) indicated an endophytic fungus belonging to the genus Fusarium, as indicated by its unpigmented, branched, and light gray hyphae.

These morphological differences reflect the potential diversity of endophytic fungi within the same plant tissue. This variation can be caused by environmental conditions, host tissue type, and the physiological response of the fungus to the growth medium. Previous research has shown that a plant's natural habitat significantly influences the diversity of microorganisms living within its tissue (Manurung et al., 2022).

Furthermore, the morphology of conidia and hyphae is closely related to the ability to produce secondary metabolites. Endophytic fungi are known to produce active compounds that mimic the metabolites of their host plants. These compounds can function as antibacterials, antioxidants, and other therapeutic agents with potential for pharmaceutical development. Microscopic examination supports the production of significant active metabolites (Monika et al., 2020).

Thus, morphological observation is not only important as an initial taxonomic step but also as an early indicator of the potential bioactivity of endophytic fungal isolates isolated from the bark of Bajakah tampala (Spatholobus littoralis Hassk.), which have significant potential for development in the pharmaceutical and modern biotechnology fields (Ismail et al., 2018).

Other identification can be done by DNA extraction (Taking genomic DNA from fungal cells), ITS PCR (Amplifying specific parts of DNA (ITS region) used for fungal identification), and sequencing Reading the nucleotide base sequence of PCR results to identify fungal species, because the results of endophytic fungi produced are not sufficient to proceed to the next stage so these tests are not carried out.

4.7. Antibacterial Activity Test

The antibacterial activity test aims to determine the potential of endophytic fungal isolates to inhibit the growth of Staphylococcus aureus bacteria. The test is conducted using the Kirby-Bauer disc diffusion method, in which paper discs soaked in the isolate solution are placed on a medium inoculated with bacteria. After 24 hours of incubation, the resulting zone of inhibition is measured as an indicator of antibacterial activity. This method is widely used in initial bioactivity testing because it is simple, rapid, and can provide initial information about the effectiveness of fungal compounds against target bacteria (Pratiwi et al., 2022).

The test results showed that all three isolates, JEB 1, JEB 2, and JEB 3, had the ability to inhibit the growth of Staphylococcus aureus, indicated by the formation of a clear zone around the disc. The results of (JEB 1) showed the highest antibacterial activity with an average inhibition zone of 27.18 mm and were categorized as very strong, while (JEB 2) had strong inhibition (17.86 mm), and (JEB 3) was in the moderate category (10.35 mm). These differences in inhibition may be due to variations in secondary metabolites produced by each endophytic fungal isolate, including compounds such as flavonoids, alkaloids, or terpenoids that have antibacterial activity (Rahayu et al., 2023).

JEB 1 even showed a greater inhibition zone effectiveness than the positive control chloramphenicol (23.42 mm), indicating that this isolate has great potential for further development as a natural antibacterial agent (Hartini et al., 2021). The resulting inhibition zone is influenced not only by the active compound's ability to kill bacteria but also by its diffusibility in agar media. The diameter of the clear zone requires testing at the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) levels (Maulidya et al., 2023).

4.8. Statistical analysis using SPSS on

The inhibition zone diameter data from three endophytic fungal isolates (JEB 1, JEB 2, JEB 3) against Staphylococcus aureus showed that the data met the requirements for parametric testing. This was evidenced by the significance values in the Shapiro-Wilk normality test and Levene's homogeneity test, both of which were greater than 0.05, at 0.122 and 0.097, respectively. Therefore, the data were considered normally distributed and homogeneous, allowing for a one-way ANOVA test. The ANOVA test showed a significance value of 0.000 ($p < 0.05$), indicating a statistically significant difference in antibacterial activity between the three endophytic fungal isolates.

This difference can be seen from the average inhibition zone shown by each isolate. Isolate (JEB 1) showed the highest antibacterial activity with an inhibition zone diameter of 27.18 ± 0.725 mm, categorized as very strong. This result even exceeded the positive control (chloramphenicol) which only produced an inhibition zone of 23.42 ± 0.640 mm. Meanwhile, (JEB 2) showed an inhibition zone of 17.86 ± 0.450 mm (strong category), and (JEB 3) of 10.35 ± 0.533 mm (moderate category). These findings indicate that the endophytic fungus JEB 1 contains the most dominant bioactive compounds that are able to inhibit the growth of S. aureus effectively so that it is continued to the MIC and MBC stages.

4.9. MIC and MBC of JEB 1

Preliminary test results indicated that JEB 1 had the highest antibacterial activity. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests were conducted using test bacteria at their optimum growth phase. In this study, JEB 1 isolate was tested against Staphylococcus aureus bacteria at various concentrations, including 1 x 106 ppm, 7.5 x 105 ppm, 5 x 105 ppm, and 2.5 x 105 ppm. The MIC test results were characterized by a cloudy medium, previously inoculated with the test bacteria and then added with JEB1 isolate, which was then incubated for 24 hours. The cloudy medium indicated bacterial growth, and it was evident that the bacteria continued to grow at a concentration of 2.5 x 105 ppm, as indicated by the cloudiness in the test tube. At a concentration of 5 x 105 ppm, Staphylococcus aureus bacteria no longer exhibited turbidity in the media, as indicated by the clear color of the test tube. This indicates that the active compound produced by the JEB 1 isolate has very high antibacterial potential. Even at a concentration of 5 x 105 ppm, the test tube appeared clear. This indicates that the MIC of JEB 1 can be specifically determined because even at a concentration of 5 x 105 ppm, bacterial growth was effectively inhibited.

The Minimum Killing Concentration (MBC) test is conducted to determine the lowest concentration of a substance that can kill a test bacterium. The MBC test uses a solid agar dilution method by dipping the suspension using a sterile loop needle from the MIC media into NA media and incubating it for 24 hours. The MBC test results showed that Staphylococcus aureus bacteria still grew in the same concentration, 2.5 x 105 ppm. The MBC results of JEB1 showed that at concentrations of 1 x 106 ppm, 7.5 x 105 ppm, 5 x 105 ppm, and 2.5 x 105 ppm, the number of colonies growing at the MBC was still countable, indicating that the bacteria were not completely killed, although their numbers decreased drastically compared to the negative control. This means that JEB 1 was effective in reducing the number of bacterial colonies, but did not achieve complete killing ability. Based on these results, the MBC of JEB 1 is likely above 1 x 106 ppm or requires a longer exposure time to achieve a full bactericidal effect (Syamsudin et al., 2021).

The combined results of the MIC and MBC tests indicate that the antibacterial activity of JEB1 depends not only on the active compound contained, but also on the concentration, duration of contact, and type of target microorganism. Isolate JEB 1 exhibited very strong bacteriostatic properties starting at a concentration of 5 x 105 ppm, and isolate JEB 1 also exhibited very strong bacteriocidal properties starting at a concentration of 5 x 105 ppm. These concentrations are important as a reference in the development of endophyte-based antibacterial agents, where understanding the specific inhibitory and killing effects forms the basis for formulating appropriate dosages for pharmaceutical applications (Hendrawan et al., 2022).

5. Conclusion

Three endophytic fungal isolates from Bajakah (Spatholobus littoralis Hassk) stems showed antibacterial activity against Staphylococcus aureus bacteria with varying levels of potency. Isolate (JEB 1) which is estimated to originate from Aspergillus sp showed very strong antibacterial activity with an inhibition zone diameter of 27.18 ± 0.725 mm. Isolate (JEB 1) had the best Minimum Inhibitory Concentration (MIC) value at a concentration of 5 x 106 ppm and the best Minimum Bactericidal Concentration (MBC) at a concentration of 5 x 106 ppm. These findings indicate that endophytic fungi, especially from Aspergillus sp, have potential as natural antibacterial agents that can be further developed as candidates for new antibacterial materials based on local natural resources.

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