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ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA FROM THE ROOTS AND LEAVES OF JAVANESE WOOD AS AN ANTIBACTERIAL SOURCE AGAINST PATHOGENS THAT CAUSE INFECTION

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Abstract:

The Javanese Wood Plant (Lannea coromandelica) is recognized for its antibacterial, antifungal, and antioxidant properties, which are beneficial in traditional medicine. This study aims to isolate endophytic bacteria from the roots and leaves of Javanese Wood and test their antibacterial activity against pathogens that cause infection. The methods used in this study include the isolation of endophytic bacteria by sterile and purification techniques, followed by the identification of bacterial morphology and biochemistry using Gram staining and testing of antibacterial activity using the disc diffusion method. The results showed that endophytic bacteria successfully isolated from the roots and leaves of Javanese Wood exhibited significant antibacterial activity against several pathogenic bacteria with varying inhibitory zone diameters. The bacterial isolates found in the roots are from the genera Pseudomonas and Bacillus, while from the leaves are found Monococcus, Bacillus, and Staphylococcus. This study concludes that endophytic bacteria from Javanese wood have the potential to be a natural source of antibacterial that can be used to overcome antibiotic resistance. The implication of this study is further development in the utilization of endophytic bacteria as an alternative antibacterial agent that is more environmentally friendly and sustainable.

Keywords: Antimicrobial, Javanese, Leaves, Roots

Introduction

Microorganisms in the natural environment comprise mixed populations of various species, including those found in soil, water, air, and food, as well as within animals and plants. Bacterial isolation is necessary to determine the species of Bacteria and study their culture, morphology, physiology, and properties. This separation technique is called "Isolation." Bacterial isolation is defined as the process of obtaining bacteria from culture media or their natural environment and cultivating them in artificial media to produce a pure culture (Bonnet et al., 2020) (Mészáros, 2025).

The principle of microbial isolation is to separate one species of microorganism from another species derived from a mixture of different types of microorganisms that exist. Touge Extract Agar (TEA) media is used to select microorganisms from the environment where it must consider factors that influence isolating microorganisms, including the type of microbial species to be isolated, the habitat or origin of microorganisms, growth media, the proper method of inoculation of microorganisms, and the method of culture of microorganisms (Wulandari et al., 2021)(Wardhani et al., 2020). Java, also known by its scientific name Lannea coromandelica, is a medicinal plant widely used in many countries, including Bangladesh, India, and Indonesia. The Buton people in Sulawesi use this plant as a traditional remedy for hematemesis, diarrhea, and scabies. Several previous studies have demonstrated that the bark and leaves of this plant possess antibacterial, antifungal, antioxidant, and analgesic properties and are known to enhance wound healing and rehabilitation in albino mice (Aldini et al., 2025)(Tedjasulaksana & Ratmini, n.d.).

Javanese Wood Plant is also a wild plant that is easy to recognize because it is used for medicinal purposes as well as a fence In fact, Javanese Wood Plant bark can be used as a natural antiseptic against fungal infections due to its chemical composition. According to phytochemical research, Javanese tree bark is said to contain alkaloids, terpenoids, steroids, saponins, flavonoids, and tannin compounds (Hidayat, 2025). The presence of endophytic bacteria on the stems and roots of Javanese Wood Plants

It is believed to exist and live in plant tissues, both in roots, leaves, stems, fruits, and twigs, by forming colonies without harming the host and spreading almost throughout vascular plants. Endophytic bacteria are one of the bioactive sources with significant potential in the fields of health, agriculture, the environment, and industry. The relationship between endophytic bacteria and their host is a mutualism, one in which the bacteria produce secondary metabolite compounds that are similar to those of the host. Therefore, it is necessary to research antibacterial compounds in endophytic bacterial extracts from L. coromandelica roots, which have the potential to be used as a medicinal agent without compromising plant populations (JAWA, 2022).

Several studies have been conducted to determine the presence of endophytic microorganisms in various plants, including rice plants (Mhd, 2025), potatoes (Firdausy et al., 2024), orchids (Janah, 2022), quinine (Pasappa et al., 2022). However, the diversity of endophytic bacteria in a plant is not always the same, as planting conditions influence it. Therefore, the exploration of endophytic bacterial diversity remains a concern. Molecular technology has also been extensively developed to analyze the presence of endophytic bacteria that cannot be cultured or grown on synthetic media (Saragih et al., 2023).

The purpose of this study is to isolate endophytic bacteria from the roots and leaves of the Javanese Wood Plant (Lannea coromandelica). It also determines the morphological and biochemical characteristics of the successfully isolated endophytic bacteria. Then identify the acquired endophytic bacteria using molecular methods or other appropriate identification methods. In addition to testing the antibacterial activity of endophytic bacteria against certain pathogenic bacteria.

Literature Review

1. Endophytic Bacteria

Endophytic bacteria are bacteria that live within plant tissues and form a symbiotic relationship with their host. Endophytic bacteria are capable of producing secondary metabolites, including bioactive substances, that are similar to those found in their host plants. The advantage of producing bioactive substances from endophytic microorganisms is that these microorganisms are easy to cultivate, have a shorter life cycle than plants, and can produce large amounts of bioactive compounds (Aqilla, 2025).

The types of endophytic bacteria in plants vary depending on the plant species and its specific environmental conditions. Endophytic bacteria live inside plants and, therefore, have a relatively stable and protected habitat. Plants can provide enough nutrients, while endophytic bacteria stimulate plant growth and suppress pathogenic microorganisms. Endophytic bacteria are beneficial to plants because they can produce siderophores, synthesize plant hormones (such as abscisic acid, indole acetic acid, and cytokinins), bind atmospheric nitrogen, and perform other important functions. Endophytic bacteria not only play a role in promoting plant growth but also contribute to the plant repair process and can produce antibiotics and bioactive compounds (Yanti, 2019).

2. Javanese Wood (Lannea coromandelicia)

Javanese wood (Lannea coromandelica) is a habit-forming plant with a woody tree habit. The taxonomic order of Javanese wood (Lannea coromandel) according to (Arifin & Rachman, 2020) is as follows:

Domain: Eukaryota

Kingdom: Plantae Phylum: Spermatophyta

Subphylum: Angiosperms Class: Dicotyledonae Order: Sapindales

Family: Anacardiaceae Genus: Lannea

Species: Lannea coromandel

Javanese wood leaves have a tapered shape with a number of 7-11 leaves, with spiral phyllotaxis (folio sparse), and are leaves that consist only of leaves and petioles**.**

Figure 1. Javanese Wood Leaf Phylotaxi

It has pinnate compound leaves arranged alternately (alternately). Each compound leaf consists of several leaflets arranged in pairs along the main stalk. It has a leaf shape in the shape of a Jurong (oval), a conical leaf tip (acuminate), a pointed leaf base (acutus),

pinnate leaf bones (penninervis), wavy leaf edges (repandus), has soft thin surface (herbaceous), bright green in color accompanied by a smooth leaf surface.

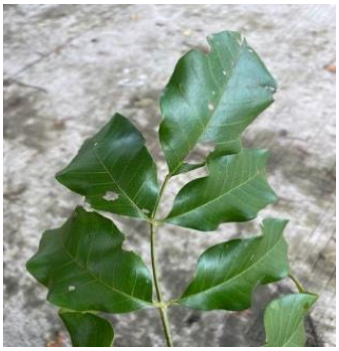

Figure 2. Javanese Wood Leaves

Javanese wood plants have a taproot system, a characteristic of dicotyledonous plants. The primary roots grow straight down with lateral root branches spreading in all directions. This root system serves to support large trees and help plants absorb water and nutrients from deeper soil layers. Where the main root structure is sturdy, long, and grows vertically into the soil, lateral roots (branch roots) spread horizontally underground, serving to increase water and mineral absorption. Secondary fibrous roots grow from the lateral roots, helping in nutrient absorption and strengthening the soil grip.

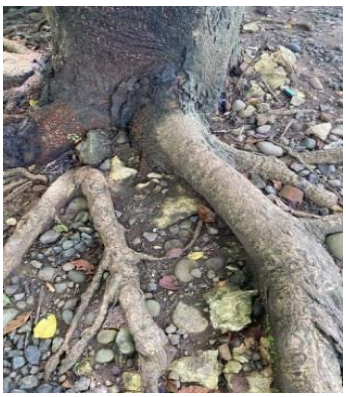

Figure 3. Javanese Wood Roots

Its taproot system allows plants to survive in soil conditions that are low in air or barren. Additionally, deep roots can prevent soil erosion and contribute to air conservation in the growing area. The roots of the Javanese Wood plant play a crucial role not only in the growth and stability of the tree but also in its ecology and potential medical benefits, which can be further explored (Noor, 2023).

3. Antibiotic Resistance and the Need for Antibacterial Alternatives

Antibiotic resistance has become one of the serious global health issues in recent decades. Pathogenic bacteria that could previously be treated with conventional antibiotics now demonstrate the ability to survive and thrive despite receiving treatment. This phenomenon is caused by several factors, including the excessive and improper use of antibiotics in medical treatment, as well as in livestock and agriculture. As a result, bacteria such as Staphylococcus aureus and Escherichia coli that are resistant to antibiotics can cause disease. Increasing antibiotic resistance is fueling the urgent need for the development of new antibacterial agents that are more effective and have a different mechanism of action than conventional antibiotics. One potential source of new antibacterial agents is endophytic microorganisms that live in symbiosis within plant tissues. These microorganisms have been shown to produce a variety of bioactive compounds, including those with antibacterial activity. The exploration of endophytic bacteria as an alternative source of antibacterial compounds can provide innovative solutions for overcoming antibiotic resistance. Bioactive compounds produced by endophytic bacteria Thus, research on the antibacterial potential of endophytic bacteria, especially those derived from plants such as Javanese wood (Nursyahid, 2022).

The types of endophytic bacteria in plants vary depending on the species and condition of the plant, as endophytic bacteria live inside plants, providing them with a relatively stable and protected habitat. Plants can provide adequate amounts of nutrients, whereas endophytic bacteria stimulate plant growth and suppress pathogenic microorganisms. Endophytic bacteria are beneficial to plants because they can produce siderophores, produce plant hormones (such as abscisic acid, indoleacetic acid, and cytokinins), bind nitrogen in the atmosphere, and perform other functions. Endophytic bacteria not only play a role in encouraging plant growth but also contribute to the plant repair process and can produce antibiotics and other bioactive compounds (Dewi et al., 2024).

4. Methods of Isolation and Identification of Endophytic Bacteria

The isolation of endophytic bacteria from plant tissues is a crucial step in the exploration of microorganisms that have the potential to produce bioactive compounds. This process aims to isolate bacteria that live inside plant tissue without being contaminated by epiphytic microorganisms on the surface. Therefore, the isolation techniques used must ensure that the bacteria obtained actually come from within the plant tissue. The isolation process of endophytic bacteria begins with taking samples (roots, stems, and leaves) of Javanese wood plants in a fresh condition, washed with running water until clean, and then cut to a size of 1-3 cm, then a surface sterilization process is carried out (FU'IKAH, 2022).

Purification is performed by transferring the growing colony into a Petri dish containing nutrient agar (NA). Once the pure culture is obtained, endophytic bacteria are stored in the NA-tilted aqua. This antibacterial activity test uses the disc diffusion method with two repetitions. The bacterial suspension is spread using sterile swabs on the Mueller Hinton Agar (MHA) media; at the time of the swab, the petri dish is rotated at an angle of 60° until the bacterial suspension is evenly distributed on the surface of the MHA. On the blank disc paper, as much as 40 µl of endophytic bacteria is dried. After incubation at a

temperature of 37° C for 24 hours, observation and measurement of the diameter of the barrier zone formed. Identification of endophytic bacteria is carried out macroscopically and microscopically. Macroscopic observations were carried out by looking at the morphology of single colonies that appeared after the streak plate method was carried out. Microscopic observations are carried out by observing the staining results. Grams using an electron microscope with a magnification of 40X (Astuty et al., 2019).

5. Anti-bacterial Activity Test

Diffusion method to be used to test antibacterial activity The diffusion method involves measuring and observing the diameter of the clear zone formed around the empty disc containing antimicrobial agents inoculated with microorganisms The antibacterial activity test was carried out three times on the leaves of the Javanese tree (Lannea coromandel) at concentrations of 0.5%, 1%, 2%, 4%, 8%, and 16% using an empty disc carried out repeatedly (Salsabila, 2020).

The purpose of using the six variations above was to determine at what concentration ethanol extract from the leaves of the Javanese tree (Lannea coromandelica) could inhibit the growth of bacteria In this test positive and negative controls were used, empty discs of antibiotic ampicillin were used as positive controls and DMSO was used as negative control Positive controls were used to control the test substance by comparing the diameter of the barrier zone formed with the test extract Ampicillin was used as a positive control because it is an antibiotic that effectively inhibits the growth of Gram-positive bacteria Ampicillin works by inhibiting the synthesis of microbial cell walls and transpeptidase enzymes, thereby inhibiting cellular biogenesis Negative controls serve as a comparator to ensure that the solvent used as a diluent does not affect the antibacterial test results of the compounds tested (Arni, 2024).

Method

Research Time and Place

This research was conducted from February to March 2025. The research took place in the Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Makassar.

Research Tools and Materials

The tools used in this study include sterile scissors/knives, sterile plastic bags, autoclaves/ovens, laminar flow, micropipettes, Petri dishes, incubators, microscopes, test tubes & tube racks, rulers/micrometers, mortars, ose needles.

The materials used in this study are the roots and leaves of Javanese wood. Ethanol 70%, 5.25% sodium hypochlorite solution, Nutrient agar (NA) media, Hydrogen peroxide (Catalase Test), Triple Sugar Iron media, agar (TSIA), Lactose Fermentation Test, Indole Motility sulfite media (Motility/ bacterial movement test), gelatin media (Gelatin Hydrolysis Test), Simmons Citrate Agar media (Citrate Test), dyeing reagents (Crystal violet, Lugol, Safranin, Acetone Alokohol) and spritus, Gram Colour Stain Set, immersion oil, protein asse K, lysozyme, Gram(+) buffer, Gram(+) buffer, Sodium Hypochlorite, nitric acid (HNO3) 0.5 M ferrous chloride (FeCl3), Amyl alcohol (C5H11OH), ammonium thiosyanate (NH4SCN) 10%.

Isolation of Endophytic Bacteria from Javanese Roots and Leaves

The roots and leaves of Javanese Wood are taken from Javanese Wood plants found in BTN Asabri, Maros Regency. The endophytic bacteria are isolated from the roots and leaves of Javanese wood in fresh conditions, The sample is weighed up to 2 grams, and the surface of the roots and leaves of the plant is sterilized by soaking the plant parts in 70% ethanol for 1 minute The soaking solution is then discarded, and the specimen is soaked in 5.25% sodium hypochlorite solution for 5 minutes and rinsed in 70% ethanol for 30 seconds After that, The roots and leaves of the plant are rinsed twice with sterile distilled water and dried on sterile filter paper After drying, cut about 2 cm from each end of each root of the plant Next, cut each root into two parts of the sterilized root ground in a mortar The dilution process is carried out from 10-4 until the dilution procedure is carried out by adding 1 ml of suspension and into the test tube containing 9 ml of distilled water Repeat the same process until the dilution series reaches 10-4 One ml sample was taken from four dilution series, namely 10-1, 10-2, 10-3, and 10-4, and inoculated into nystatin-containing agar (NA) Culture media containing the sample was incubated for 2 x 24 hours and observed until colony growth Pure endophytic bacterial isolates were morphologically identified based on colony color, colony edge shape, colony height and consistency, and the growth rate of the colony.

Gram Staining

Gram Staining Performed to observe microscopic characteristics Gram staining was performed on 2 x 24-hour-old bacterial cultures obtained from pure bacterial isolates After processing, the dried specimen can be examined under a microscope When staining indicates the bacteria are red, then the bacteria are gram-negative If the bacteria are purple, they are gram-positive bacteria Morphological Identification

Identification of colony morphology was performed to observe macroscopic characteristics. Endophytic bacterial cultures were inoculated into NA media using OSE lines and needles Incubation at 370 °C for 24 hours Optical properties of endophytic bacteria include colony shape, colony edges, colony color, and colony surface (height)

Motive Test

The purpose of this test is to determine the mobility of the bacteria The needle hole is used to collect one round of endophytic bacterial isolates and inoculate it by piercing a vertical plate of indole sulfur motility medium (SIM) Then, incubated at a temperature of 44-37°C for 48 hours

Citrate Test

The purpose of this test is to find out if bacteria use citric acid as a source of carbon. One ose isolate of endophytic bacteria was collected using lose clinician, inoculated into Simmons citrate agar (SCA) media, and incubated at a temperature of - 370 °C for 48 hours The change in color from green to blue suggests that bacteria can use citric acid as the sole source of carbon

Gelatin Hydrolysis Test

The purpose of this test is to determine the ability of bacteria to produce gelatinase enzymes. Using a needle, take one isolate of endophytic bacteria and inoculate the gelatin media by making a hole. Then, incubate at a temperature (temperature 370°C) for 48 hours. Positive results will be obtained if the gratin in the medium is soluble. That is, the bacteria can produce gratinase enzymes. Additionally, freezing the media will yield negative results.

Sugar Fermentation Test (TSIA)

The purpose of this test is to determine the ability of bacteria to ferment carbohydrates Using a circular needle, take one circle of bacterial isolate And inoculate into Triple Sugar Iron Agar (TSIA) Next, take one more round of bacterial isolate and rub it on the surface of the medium Then, incubate at 37°C for 48 hours The change that occurs after incubation is that the medium changes color to yellow indicating acidity, red indicates alkalinity, and black indicates the production of H2S (hydrogen sulfide) If the medium rises, it indicates microorganisms are capable of producing gases.

Catharlase test

This test helps identify groups of bacteria that can produce the catalase enzyme The catalase test is performed using 1 ose isolate to collect the isolated bacteria and dip them in a test tube containing the reagent hydrogen peroxide (H2O2) Positive results are indicated by the formation of bubbles and negative results are indicated by the absence of bubbles Data Analysis

The results of the antibacterial activity test will be described based on the size of the barrier zone formed. This data was then analyzed to determine the correlation between the type of endophytic bacteria and the level of Effectiveness in inhibiting the growth of pathogenic bacteria. Statistical data processing, such as ANOVA, can be used to compare differences in antibacterial activity between root and leaf samples, as well as between different bacterial isolates. Based on the results of antibacterial tests, endophytic bacteria from the roots and leaves of Javanese wood plants that have large inhibition zones will be considered potential candidates for the development of antibacterial drugs.

Results and Discussion

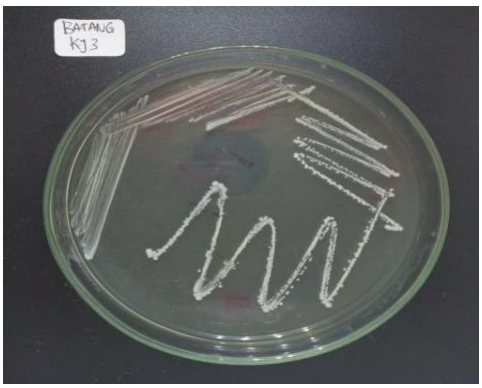

Figure 4. Isolate of endophytic bacteria of root origin of Javanese Wood Plant

The results of isolating endophytic bacteria from the roots and leaves of the Javanese Wood plant yielded a single colony. Isolation was carried out on NA media to maintain the purity of bacterial cultures. The results of the analysis of the isolated colony structure, obtained from both roots and leaves, revealed the colony's structure. The results of isolating and identifying endophytic bacteria from the roots and leaves of the Javanese Wood plant were then biochemically tested. The isolates obtained are characterized by

bacterial cells through Gram staining and determination of the presence of spores. The characterization of colonies consists of the shape, color, margin, nature, and texture of the colony. The results of various biochemical tests on the isolate are characterized according to Bergey's Manual. Some isolates showed positive results on biochemical tests.

Discussion

Research and investigation were conducted at the Department of Biology, FMIPA, State University of Makassar, to identify the types of endophytic bacteria isolated from the roots and leaves of Javanese wood plants through bacterial isolation and characterization. Endophytic bacteria inhabiting the roots of the Javanese wood plant were identified in two isolates, specifically from the genera Pseudomonas sp. and Bacillus sp. In contrast, endophytic bacteria found on the stems of Javanese wood plants include Monococcus sp., Bacillus, and *Staphylococcus sp.*

Endophytic bacteria can enter plant tissues generally through the roots, but parts of plants that are exposed to direct air, such as flowers, stems, leaves (through stomata), and cotyledons, can also be an entry route for endophytic bacteria. Endophytic bacteria that have entered the plant can either grow at a single specific point or spread throughout the plant. These microorganisms can live within vascular vessels or in intercellular spaces, roots, stems, leaves and fruits (Simarmata et al. 2007; Bacon & Hinton 2006).

The results of this study show that endophytic bacteria are found in the roots and leaves of the Javanese Wood plant. Further tests such as biochemical and microbiological tests carried out on the 4 isolates of endophytic bacteria showed that the endophytic bacteria that inhabit the roots of the Javanese Wood plant were from the genus Pseudomonas and Bacillus while the endophytic bacteria found in the stems of the Javanese Wood plant were from the genus Monococcus, Bacillus and Staphylococcus.

The data obtained indicate that endophytic bacteria found on the stem are also present in the roots, specifically Bacillus. The number of endophytic bacteria in plants cannot be determined with certainty, but these bacteria can be detected by isolating them in agar media. The agar medium used to isolate endophytic bacteria in this study is nutrient agar (NA).

Conclusion

This study successfully isolated endophytic bacteria from the roots and leaves of the Javanese Wood Plant (Lannea coromandelica), including the genera Pseudomonas sp., Bacillus sp., Monococcus sp., and Staphylococcus sp. The morphological and biochemical characterization of endophytic bacteria is conducted through Gram staining and various biochemical tests, including catalase, motility, citrate, and gelatin hydrolysis tests, which reveal the diverse properties of these microorganisms. In addition, antibacterial activity tests demonstrated the potential of endophytic bacteria as a source of antibacterial agents, with the formation of significant barrier zones against certain pathogenic bacteria. The results of this study confirm the importance of exploring endophytic bacteria for the development of environmentally friendly antibacterial drugs that do not threaten the sustainability of plant populations.

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