POTENTIAL OF ENDOPHY BACTERIAL ISOLATE FROM LEAF OF CHINESE TEMPERATURE (Cassia alata L) ON THE GROWTH OF THE BACTERIA OF STEPHYLOCOCOCUS AURUS

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ABSTRACT

This research aims to To determine the effect of endophytic bacterial isolates from the leaves of the Chinese Ketepeng plant (Cassia Alata L) on the growth of Staphylococcus aureus and to determine the significant difference between each treatment of endophytic bacterial isolates from the leaves of the Ketepeng Cina (Cassia Alata L) plant against Staphylococcus aureus.
This research method used a completely randomized design (CRD) with 4 treatments and 6 replications. The treatments were: BEKC 1 (Chinese Ketepeng Endophytic Bacteria 1), BEKC 2 (Chinese Ketepeng Endophytic Bacteria 2), Positive Control (Chloramphenicol), and Negative Control (Aquades).
The results obtained two isolates of endophytic bacteria, namely isolates BEKC 1 with an inhibition zone of 9mm included in the medium category, and isolates BEKC 2 with an inhibition zone of 10mm included in the strong category. Analysis resultthat there was an effect of endophytic bacterial isolates from the leaves of the Chinese ketepeng plant (Cassia alata L) on the growth of Staphylococcus aureus bacteria. Then from the results of further tests using Duncan's test, it was found that each treatment, namely the negative control treatment was significantly different from BEKC 1, BEKC 2 and positive control, while there was no significant difference between BEKC 1, BEKC 2 and positive control.

Keywords: Endophytic Bacteria, Chinese Ketepeng (Cassia alata L), Staphylococcus aureus

INTRODUCTION

Infectious diseases are one of the main public health problems in developed and developing countries. The World Health Organization (WHO) states that this disease is the main cause of death in children. WHO data in 2012 stated that the mortality rate of children <5 years in Indonesia was caused by infectious diseases with a percentage of 1-20% [11].

The spread of this infection source can be through various intermediaries or known as vectors, namely air, animals, objects, and also humans themselves. Even without realizing the hospital is a high risk place as a source of transmission. Infectious diseases are diseases caused by the entry and proliferation of microorganisms, a broad group of microscopic organisms consisting of one or many cells such as bacteria, fungi, parasites and viruses [16].

Infectious diseases occur when the interaction with microbes causes damage to the host body and this damage causes various clinical symptoms and signs.

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Microorganisms that cause disease in humans are called pathogenic microorganisms, one of which is pathogenic bacteria [18].

The bacterium Staphylococcus aureus (S. aureus) is said to be the most common cause of nosocomial infections, namely infections acquired by patients after hospital admission. Several types of diseases that can be caused by S. aureus infection are respiratory tract infections, impetigo, abscesses, toxic shock syndrome, and food poisoning with symptoms such as nausea, vomiting, and diarrhea, mastitis, dermatitis (skin inflammation) [6].

Antimicrobials are substances or drugs used to eradicate microbial infections in humans. The term means "against life". In other words, antibiotics are substances produced by living organisms (microorganisms), which can inhibit the growth of other microorganisms, and can even destroy them [2].

Antimicrobial Properties

Bacteriostatics, namely substances or materials that can inhibit or stop the growth of microorganisms (bacteria). Under these conditions the number of microorganisms becomes stationary, can no longer multiply and reproduce.

Bacteriocides, substances or materials that can kill microorganisms (bacteria). In this case the number of microorganisms (bacteria) will be reduced or even exhausted, can no longer do multiplication or reproduce.

Antimicrobial Mechanism of Action Antimicrobials have the following mechanism of action:

Inactivation of certain enzymes

Inactivation of certain enzymes is a common mechanism of antiseptic and disinfectant compounds, such as aldehyde derivatives, amides, carbanilides, ethylene oxides, halogens, mercury compounds and quaternary ammonium compounds.

Protein Denaturation

Alcohol derivatives, halogens and halogenators, mercury compounds, per-oxides, phenol derivatives and quaternary ammonium compounds work as antiseptics and disinfectants by denaturing and conjugating bacterial cell proteins.

Changes the permeability of the bacterial cytoplasmic membrane

This method is a working model of amine and guanidine derivatives, phenol derivatives and quaternary ammonium compounds. By changing the permeability of the bacterial cytoplasmic membrane, these compounds can cause the leakage of essential cell constituents, resulting in the death of bacteria.

Some dyes, such as triphenylmethane derivatives and acridine derivatives, work as antibacterials by binding strongly to nucleic acids, inhibiting DNA synthesis and causing changes in the skeleton of mutations in protein synthesis.

Formation of chelate

Some phenol derivatives, such as hexachlorophene and oxyquinoline can form chelates with Fe and Cu, then the chelated form enters the bacterial cell. High levels of metal ions in cells cause disruption of the function of enzymes, so that microorganisms die.

Acts as an antimetabolite

Antimicrobials work by blocking specific metabolic steps of microbes, such as trimethoprim and sulfonamides, which block important enzymes of folate metabolism. Inhibition of cell wall synthesis

This class of antimicrobials can inhibit the synthesis or inhibit the activity of enzymes that can damage the cell walls of microorganisms. Included in this group are: penicillins, cephalosporins, vancomycin, cycloserine, bacitracin.

Inhibition of cell membrane permeability function. Antimicrobials act directly on cell membranes that affect permeability and cause the release of

intracellular compounds of microorganisms.

Inhibition of protein synthesis

Antimicrobials affect the function of the ribosomes in microorganisms causing inhibition of protein synthesis.

Nucleic acid inhibition

Agents that affect bacterial nucleic acid metabolism, such as para rifamycins for example, rifampin and rifabutin which inhibit RNA polymerase, and quinolones, which inhibit topoisomerase

Antimicrobial Resistance

Resistance is the resistance of a microorganism to a particular antimicrobial. The cause of microorganism resistance is the use of inappropriate antimicrobials, for example the use of inadequate doses, irregular or discontinuous use, as well as treatment time that is not long enough, so to prevent or slow down the occurrence of resistance, the use of antibiotics is necessary. noticed.

A microorganism can be resistant to a drug by one or more of the following mechanisms:

Produces paralyzing enzymes

Among these enzymes include beta-lactamases (penicillases) which hydrolyze penicillin and transferase enzymes that inactivate aminoglycosides. Changes in the structure of the receptor or target molecule

This includes changes in ribosomal components required in infection such as erythromycin and aminoglycosides

Changes in drug permeability

Tetracyclines are capable of accumulating susceptible microorganisms, but not resistant microorganisms. Altering metabolic pathways establish alternative metabolic shortcuts. It can occur in bacteria that are resistant to sulfonamides, and fungi that are resistant to flucytosine.

Altering the amount of drug response Some microorganisms become resistant to trimethoprim by synthesizing large amounts of the enzyme dehydrofolate reductase, which is the goal of the drug's action.

Decreases the affinity of the receptor for the drug

Resistance to aminoglycosides may be related to the loss or alteration of specific proteins on the bacterial 30S ribosome

Microorganism growth

Growth can be defined as an increase in the number or volume and size of cells. The growth curve of microorganisms can be separated into four main phases, namely the lag phase (adjustment), the growth phase (exponential phase), the maximum stationary phase, and the population decline phase (death) [13].

Microorganism growth phase

The growth phase of microorganisms can be divided into 4 phases, namely lag (adjustment), log (exponential), stationary and decline (death) [13].

Lag phase (adjustment).

The adjustment phase is a phase in which cells, deprived of metabolic and enzyme properties due to unfavorable conditions in the previous culture, adjust to their new environment.

The hallmark of the lag phase is that there is no increase in the number of cells, only an increase in cell size [19].

Log phase (exponential)

During the exponential phase, the cells are in a stable state, but new cell material is formed at a constant rate, but the new material itself is catalytic so the mass increases exponentially. This continues until one of two things happens: one or more of the nutrients in the seed are depleted, or toxic metabolic products accumulate so that growth is stunted.

Stationary phase

The growth of microorganisms stops and a balance occurs between the number of dividing cells and the number of dead cells. The depletion of nutrients or the accumulation of toxic metabolic products will cause growth to stop altogether. In

most cases, however, cell turnover results from slow cell loss due to death, which is offset by the formation of new cells through growth and division. When this happens, the total number of cells will increase slowly even though the number of living cells is constant.

Decline Phase (death phase)

After some time in the stationary phase, which varies for each type of organism and state of the culture, the mortality rate increases until it reaches a stable level. after most cells die, the rate of death decreases drastically, so the small number of surviving cells can add up for months or even years. This in some cases signifies cell turnover, some cells grow with nutrients released by cells that die and undergo lysis.

Relationship of Inhibitory Zone Formed with Microorganism Growth.

The inhibition zone is a clear zone around the paper disk which indicates the inhibition of the growth of microorganisms due to the activity of secondary metabolic compounds. The growth of microorganisms can be seen from the diameter of the inhibition zone formed and the shape of the inhibition zone around the paper disk

Endophytic mold isolates on parijoto leaf plants (Medinilla speciosa Blume) can inhibit bacterial growth with an average diameter of the inhibition zone produced by 5-10 mm mold which is included in the moderate inhibition category, meaning that the growth of microorganisms is not completely inhibited. Besides being seen from the diameter of the inhibition zone, the growth of microorganisms can also be seen from the shape of the inhibition zone formed around the paper disk [3].

The shape of the inhibition zone formed around the paper disk indicates the inhibition of the growth of microorganisms or there is no inhibition of the growth of microorganisms at all. The readings of the experimental results of microorganism growth are based on three categories of inhibition zones around the paper disk, namely: (1) total inhibition zone: when the inhibition zone formed around the paper disk looks clear, (2) partial inhibition zone: if it is inside the inhibition zone that is formed, the growth of several colonies was still visible, and (3) zero inhibition zone: if there was no inhibition zone formed around the paper disk [3].

Anti-Bacterial

Definition

Antibacterial is a substance or compound that can suppress or kill the growth or reproduction of bacteria [10].

Antibacterial Resistance Test

Resistance test is a test carried out to determine the sensitivity of bacteria to an antibiotic.

Resistance problems occur when bacteria change bacteria change which causes a decrease or loss of effectiveness of drugs, chemicals or other chemicals used to prevent or treat infections. The main cause of antibiotic resistance is its widespread and irrational use [28].

Antibacterial Mechanism of Action

Based on its mechanism of action in inhibiting the growth of microorganisms [22]. Antibacterial is classified as follows: Antibacterial that can inhibit cell wall synthesis. The bacterial cell wall is very important to maintain the structure of the bacterial cell. Therefore, substances that can damage the cell wall will lyse the cell wall so that it can affect the shape and structure of the cell, which in turn can kill the bacterial cell.

Antibacterial that can disrupt or damage cell membranes Cell membranes have an important role in regulating the transport of nutrients and metabolites that can enter and leave the cell. The cell membrane also functions as a place for respiration and biosynthetic activity in cells. Some types of antibacterial can

disrupt cell membranes so that it can affect the life of bacterial cells.

Antibacterial that can interfere with nucleic acid biosynthesis. The process of DNA replication in cells is a very important cycle for cell life. Several types of antibacterials can interfere with the metabolism of these nucleic acids, thus affecting all phases of bacterial cell growth.

Antibacterial that inhibits protein synthesis. Protein synthesis is a series of processes consisting of a transcription process (ie DNA is transcribed into mRNA) and a translation process (ie mRNA is translated into protein). Antibacterial can inhibit these processes will inhibit protein synthesis the criteriafor antibacterial strength are as follows [11].

1) Inhibition zone diameter > 20 mm : very strong inhibition
2) Inhibition zone diameter 10-20 mm: strong inhibition
3) Inhibition zone diameter 5-10 mm: medium inhibition
4) Inhibition zone diameter 0-5 mm: weak inhibition

Treatment that can be done to treat diseases caused by bacteria is to use antibiotics. Antibiotics are drugs that require special attention in their use. The use of these drugs has increased in the last few decades [28].

Handling of infection is done after getting the infecting bacteria. Some examples of antibiotics given are amoxicillin for infections by Gram-positive bacteria, chloramphenicol in infections by Gram-positive and Gram-negative bacteria, clindamycin for infections by Gram-positive bacteria, penicillin in infections by group A Streptococcus, Treponema pallidum, and Neisseria. meningitidis, sulfadiazine in urinary tract infections by Escherichia coli, Klebisella, and Proteus mirabilis [17].

Long-term use of antibiotics can cause new health problems such as impaired liver function, decreased white blood cell count, allergies, acute and chronic poisoning, and other health effects. Antibiotics can also cause resistance so that disease treatment requires higher antibiotic doses [12].

Of all the antibiotics available, the -lactam group is the drug most often used in the treatment of bacterial infections. The level of bacterial resistance to -lactams continues to increase over time and covers the whole world. The -lactamase enzyme is the main cause of -lactam resistance, especially in gram-negative people [7].

To avoid the large side effects of using synthetic antibiotics and resistant cases that have occurred in many synthetic antibiotics, new antibiotics are developed from natural ingredients by utilizing bacterial isolates, one of which is endophytic bacteria. Endophytic bacteria are bacteria that live in host plant tissues without causing disease symptoms. Endophytic bacteria enter plant tissues generally through roots, but plant parts that are exposed to direct air such as flowers, stems and cotyledons, can also be an entry point for endophytic bacteria [5].

Plants that have potential as antibacterial include Chinese ketepeng. The Chinese Ketepeng plant has been used as a remedy for worms, canker sores, constipation, tinea versicolor, ringworm, scabies and itching. Chinese Ketepeng leaves can treat constipation, syphilis, diabetes, intestinal parasitosis and hernia [1]. This is due to the chemical content contained in it. Chinese ketepeng leaves contain several chemical compounds, namely alkaloids, tannins, chrysophanic acid, glycoside compounds, aloeemodina, bitter substances, tanning substances [15]. and flavonoids. Furthermore, Chinese Ketepeng (Cassia Alata L.) can be used as a traditional medicine due to the chemical

content contained in it such as rein aloe emodina, rein aloe emodina diantron, chrysophanic acid (dehydroxymethyl antroquinone), tannins, alkaloids, and flavonoids [14]. Flavonoids in plants have anti-inflammatory, anti-allergic, antimicrobial, antioxidant effects, and are effective against several groups of fungi and bacteria. The activity of Ketepeng extract as an antibacterial has been proven by several research results. That the ethanol extract was able to inhibit the growth of bacteria that cause canker sores was characterized by a reduction in the number of colonies at different extract concentrations [15]. At a concentration of 2.5% the Chinese ketepeng leaf extract was better at inhibiting the growth of the diameter of the fungus Phytophthora Palmivora, namely 30.18% and after being analyzed using the linear regression equation method, the concentration of 2.5% had the greatest y value, which was 30.48% [26]. The activity of Ketepeng extract as an antibacterial has been proven by several research results. That the ethanol extract was able to inhibit the growth of canker sores-causing bacteria was indicated by a reduction in the number of colonies at different extract concentrations [15]. At a concentration of 2.5% the Chinese ketepeng leaf extract was better at inhibiting the growth of the diameter of the fungus Phytophthora Palmivora, namely 30.18% and after being analyzed using the linear regression equation method, the concentration of 2.5% had the greatest y value, which was 30.48% [26]. The activity of Ketepeng extract as an antibacterial has been proven by several research results. That the ethanol extract was able to inhibit the growth of bacteria that cause canker sores was characterized by a reduction in the number of colonies at different extract concentrations [15]. At a concentration of 2.5% the Chinese ketepeng leaf extract was better at inhibiting the growth of the diameter of the fungus Phytophthora Palmivora, namely 30.18% and after being analyzed using the linear regression equation method, the concentration of 2.5% had the greatest y value, which was 30.48% [26].

Sampling of Chinese ketepeng leaves on plant parts, especially on the leaves, because in the leaves there is a photosynthesis process where the photosynthesis process is the process of making food by green plants through biochemistry on chlorophyll with the help of sunlight with the results of the photosynthesis process by causing endophytic bacteria. will be found more in the leaves. Because in that section there is a source of food and energy for endophytic bacteria to breed.

RESEARCH METHODS

This research was carried out in a laboratory experimental manner using a quantitative descriptive approach.

Data collection techniques used in this study are laminar airflow, oven, incubator, autoclave, Erlenmeyer, test tube, petri dish, object glass, centrifuge, shaker incubator, ose needle, microscope, analytical balance, Bunsen, aluminum foil. , measuring glass, glass stirrer, tweezers, caliper, tissue, cotton and camera.

The materials needed for the study were Chinese ketepeng leaves (Cassia Alata L), aquades, spritus, paper discs, 90% ethanol, 1% NaOCI (Sodium Hypochlorite) solution, Crystal violet, safranin, lugol, and the test bacteria used Staphylococcus Aureus, media NA (Nutrient Agar) control is positive (+).

In this research, what I did were:

1. **Tool Sterilization**

   Glass utensils that will be used in the study, such as petri dishes, Erlenmeyer, test tubes, measuring cups and glass objects are washed with laundry soap containing antiseptic ingredients and then dried. After

drying, the glassware was wrapped in paper and aluminum foil and placed in the oven at 1700 C for 1 hour. Ose and tweezers are sterilized by burning over a Bunsen flame

2. **Harvesting Plants/Leaves of Chinese Ketepeng (cassia alata L)**

The sample used to isolate endophytic bacteria was the Chinese ketepeng leaf (cassia alata L) which was obtained from the Bone Bolango Regency, Gorontalo Province, the leaves that were determined as samples were located on the branches in one-third of the plant from the top, namely the final shoots (leaves 3-4) and previous shoots (leaves 5-6) which have been physiologically perfect. Leaf collection from each plant was carried out after harvest.

3. **Preparation of Nutrient Agar (NA)**

Making NA media (Nutrium Agar) based on the provisions on the packaging and the number of petri dishes to be used, namely by weighing 2.05 grams of NA media and adding 75 ml of distilled water. The media is then heated with a water bath (hot plate) until it boils. Furthermore, the media was sterilized using an autoclave at a temperature of 1210 C with a pressure of 15 pounds for 15 minutes. The sterile media was poured into a petri dish aseptically and allowed to solidify at room temperature

4. **Isolation of Endophytic Bacteria from Chinese Ketepeng Leaves (Cassia Alata L)**

Endophytic bacteria isolated from Chinese ketepeng leaves *(Cassia Alata L).*Chinese ketepeng leaves were washed using running water for 5 minutes. After washing, the surface was sterilized by inserting Chinese ketepeng leaves into 70% ethanol solution for 2 minutes, followed by immersion in 1% NaOCl solution for 2 minutes and finally soaked in sterile distilled water for 1 minute. After that, it is cut to the required size and then affixed to a petri dish that already contains NA media*(Nutrient Agar).* Observations were made every day until the growth of bacteria was visible. Then the endophytic bacteria were isolated and purified on new NA media. The endophytic bacteria used for the study were bacteria that grew around pieces of Chinese ketepeng leaves.

5. **Endophytic Bacteria Purification**

The medium that will be used in the purification of endophytic bacteria is NA (Nutrient Agar) medium. Endophytic bacteria grown on NA medium were purified on flat media and NA inclined media, respectively. Then it was incubated for 24-48 hours at a temperature of 250C. After incubation, the shape and color of the colonies were observed on NA medium, each colony with different shapes and colors was subcultured on the new NA medium.

6. **Observation of Endophytic Bacteria Morphology**

The growing bacteria were observed for their morphological characters including the shape of the colony, the surface of the colony and the color of the colony. Observation of cell morphology was based on the gram staining method.

Gram staining procedure, in which bacterial isolates were taken 1 ose and scratched on the surface of sterile preparations and then fixed. 1 drop of crystal violet was added to the surface of the preparation containing the bacterial layer and allowed to stand for 1 minute. After 1 minute, the preparations were rinsed with water until the dye faded. The preparations were dried over a spirit fire. After drying, 1 drop of iodine solution was

added to the surface of the preparation and allowed to stand for 1 minute. After 1 minute, the preparations were rinsed with water. After that, the preparations were rinsed with 70% ethanol until all the dye faded and then washed with water. The preparations were dried over the fire of the syringe. After drying,

7. Antimicrobial Potential Test

Antimicrobial potency test is carried out through several stages, namely:

Cultivation

Growing isolates found on NB media. Colonies of endophytic bacteria that have been incubated on NB medium for 1x24 hours at a temperature of 370C, one loop is taken using an ose needle and grown on NB medium in an Erlenmeyer with a volume of 50 ml, at 37oC for 1x24 hours using a 130 rpm shaker for 1x24 hours. .

Antimicrobial Isolation

After completion of cultivation, each medium was centrifuged at a speed of 3800 rpm at 40C for 20 minutes. The resulting supernatant was then used in testing the antibacterial activity against Staphylococcus aureus [27].

Antimicrobial Test Against Staphylococcus aureus

The medium used for the antibacterial activity test was NA medium. The antimicrobial activity test was carried out using the Kirby-Bauer test method using disc paper. The disc paper used was immersed in the supernatant for 30 minutes.

The disc paper was taken using sterile tweezers and placed on the antimicrobial activity test medium, then incubated for 18-24 hours at 37°C. After the incubation period is complete, observations are made on the clear zone or inhibition zone formed and the diameter is measured. Samples that have the potential to produce antibacterial substances are indicated by the presence of a clear zone or an inhibition zone [19].

Observation of the inhibition zone formed can be seen from the shape of the area formed. If the diameter of the inhibition zone is 5 mm or less, the inhibitory activity is categorized as weak, the diameter of the inhibition zone is 5-10 mm, then it is categorized as moderate, and if the diameter of the inhibition zone is 10 mm or more, the inhibitory activity is categorized as strong [9].

8. Barrier Zone Measurement

Observations were made after 24 hours of incubation. The inhibition zone formed around the disc was measured vertically and horizontally in mm using a caliper [3].

Technical analysis of data obtained from SPSS statistics. The research data will be analyzed using analysis of variance (ANOVA) with the Kruskall-Wallis test to test whether there is an effect of endophytic bacterial isolates on the growth of Staphylococcus aureus.

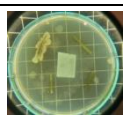
If there is an effect, it will be continued with Duncan's test to see the significant difference between each treatment.

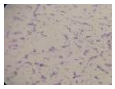
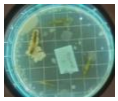
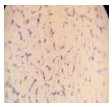
RESEARCH RESULT

Endophytic Bacteria Isolation Results

Based on the results of the study, 2 isolates of endophytic bacteria were successfully isolated from the leaves of the Chinese Ketepeng (Cassia alata L) plant, as shown in Table 1.

Table 1 The results of endophytic bacterial isolates from the leaves of the Chinese ketepeng plant

NO	Isolate	Characteristics	Picture
1	BEKC 1	a. Morphological Features 1. Yellow 2. Curled	

		3. Corrugated colony edge 4. Smooth surface	
		b. Gram stain 1. Gram Positive 2. Purple 3. Basil shaped	 Coloring
2	BEKC 2	a. Morphological Features 1. Slightly white clear 2. circular shape 3. Flat edge	
		b. Gram stain 1. Gram positive 2. Purple 3. Cocci shape	 Coloring

Personal source 2021

Potential Test Results of Endophytic Bacteria Isolates from Chinese Ketepeng (Cassia alata L) Plant Leaves on the Growth of Sthapylococcus aureus

Based on the results of the research that has been carried out, data were obtained regarding the effect of endophytic bacterial isolates on the growth of Staphylococcus aureus. The results of the average diameter of the inhibition zone that have been treated obtained data as shown in (Table 2).

Table 2 Average Inhibitory Zone Diameter (mm) Endophytic Fungus Isolate Chinese ketepeng leaf as antibacterial

No	Test Group	Average (mm)	Category
1	Negative Control (-)	0	-
2	BEKC 1	9	Currently
3	BEKC 2	10	Strong
4	Positive Control (+)	12	Strong

Personal source 2021

Note:
BEKC = Chinese Ketepeng Endophytic Bacteria
Control (+) = Chloramphenicol
Control (-) = Aquadest

Based on Table 2, it can be seen that the average diameter of the inhibition zones in the positive control group and BEKC 2 group was larger than the BEKC 1 and negative control group. Based on the results of the prerequisite tests that have been carried out, it can be stated that parametric tests cannot be carried out because in the normality and homogeneity tests there are no significant results with results 0.003 meaning that the results are not homogeneous and not significant with results 0.006, 0.212, 0.415 not normality and 0.002 not homogeneous. Based on the decision making of the homogeneity test where if the significant value is 0.05 then the value is homogeneous but if the value is 0.05 then the value is not homogeneous. The results of this test produce a significance value of P = 0.000 or < 0.

After the analysis of hypothesis 1 test it was known that there was an effect of endophytic fungal isolates on the growth of Staphylococcus aureus, then further tests were carried out, namely the Duncan test. Duncan's test aims to analyze hypothesis 2, namely to determine the significant difference between each treatment of endophytic isolates in inhibiting or killing the growth of Staphylococcus aureus.

Table 3 Results of Analysis Using Duncan's Test on Each Treatment

No	Group Test	Average (mm)	Symbol
1	Negative controlF (-)	0	A
2	BEKC 1	9	B
3	BEKC 2	10	B
4	Positive Control (+)	12	C

Personal source 2021

Note:
The eye symbols (letters) that are not the same show a significant difference

Based on Table 3, it can be seen that the negative control treatment was significantly different from BEKC 1, BEKC 2 and positive control, while the positive control was significantly different from BEKC 1, BEKC 2 and negative control. Meanwhile, there was no significant difference between BEKC 1 and BEKC 2 isolates. DISCUSSION

Endophytic bacteria are microscopic microorganisms that live in healthy plant tissues such as leaves, roots, fruits, seeds and stems [24]. Endophytic bacteria have the potential to be used as a producer of secondary metabolites such as those contained in their host plants. Endophytic bacteria enter plant tissues generally through roots or other parts of plants [24].

One of the plants that are used as medicine is the Chinese ketepeng. People use Chinese ketepeng as a remedy for tinea versicolor, scabies and ringworm. The ethanol extract of Chinese ketepeng leaves contains antibacterial and antifungal compounds, namely tannins, saponins, alkaloids, steroids, terpenoids, flavonoids, and antarquinone. Research on ethanol extract of Chinese ketepeng leaves is able to inhibit the growth of staphylococcus aureus.

Furthermore, the alcohol extract of Chinese ketepeng was able to inhibit the growth of microbes Escherichia coli, Bacillus subtilis, Salmonella typhi, Pseudomonas aeroginosa, and Staphylococcus aureus [14].

Testing the potential of endophytic bacterial isolates as antimicrobials using the supernatant from the centrifugation of endophytic bacterial isolates from the leaves of the Chinese ketepeng plant (Cassia alata L) which have been cultivated to produce secondary metabolites. Secondary metabolites are compounds synthesized by microbes, not to fulfill their primary needs but to maintain their existence in interacting with their environment. Secondary metabolites are produced by microorganisms at the end of the stationary phase of their growth. This is because secondary metabolites are usually synthesized at the end of the cell growth cycle, namely in the stationary phase when the population remains due to the number of dead cells [19].

Endophytic bacteria from Chinese ketepeng leaves are included in endophytic microbes that produce antibiotics, by endophytic Streptomyces sp. Based on the mechanism, endophytic bacteria produce antibiotics by inhibiting cell wall synthesis. The microbial cell wall is chemically peptidoglycan, which is a complex of mucopeptide polymers (glycopeptides). The structure of the cell wall can be damaged by inhibiting the reaction of its formation or changing it after the cell wall has been formed.

Antimicrobials can inhibit synthesis or inhibit the activity of enzymes such as transpeptidase enzymes that can cause cell wall damage which results in cell lysis.
Based on macroscopic observations, it can be seen that the isolation of endophytic bacteria from Chinese ketepeng leaves obtained 2 isolates of endophytic bacteria that have different colony morphological characteristics (Table 1) for isolate code BEKC 1 having morphological characteristics of yellow curlet-shaped, wavy colony edges, and smooth surface. BEKC 2 isolates had white morphological characteristics in the form of circular jagged colony edges, and wrinkled surfaces. Furthermore, in addition to observing the morphology of endophytic bacterial colonies, observations were also made with the morphology of endophytic bacterial cells using the gram staining method.The color difference of microbial colonies is influenced by the intracellular pigments produced by these microbes,

including carotenoid pigments, anthocyanins, melanin, tripyrylmethene and phenazim. Which will give different colors to each colony. [23]

Based on the observation of the morphology of endophytic bacterial cells using the Gram staining method, it was found that the BEKC 1 isolate referred to in Gram positive bacteria was purple in color and was in the form of a bacillus. while the BEKC 2 isolates included in the gram-positive bacteria were purple in color and shaped like cocci.

Basil is a bacterium that has the shape of a short stick or small rod and is cylindrical. Basil can be coupled in length, coupled in twos, or separated from each other. While cocci are bacteria that have a round shape like small balls. Groups of bacteria in the form of cocci live in swarms and in pairs in the form of colonies [29].

Furthermore, the antibacterial activity test against bacteria was carried out *Staphylococcus aureus.* To BEKC 1 isolates had an inhibition zone of 9 mm in the medium category, while BEKC 2 isolates had an inhibition zone of 10 mm in the strong category. If the inhibition zone formed in the agar diffusion test is less than 5 mm in size, then the inhibitory activity is categorized as weak. If the zone of inhibition measuring 5-10 mm is categorized as moderate, 10-20 mm is categorized as strong and 20 mm or more is categorized as very strong [26].

Based on the results of this analysis using the Kruskal Wallis method. the result of significance < value: 0.05, it can be concluded that H0 is rejected and H1 is accepted. Based on the average value of the inhibition zone obtained from the measurement results on Staphylococcus aureus. Furthermore, further test analysis was carried out using Duncan's test. The test results showed that the negative control treatment was significantly different from BEKC 1, BEKC 2 and positive control, while the positive control was significantly different from BEKC 1, BEKC 2 and negative control. Meanwhile, there was no significant difference between BEKC 1 and BEKC 2 isolates. The inhibition of Staphylococcus aureus growth activity was thought to be caused by the ability of endophytic bacteria isolates to produce secondary metabolic compounds. The secondary metabolites referred to are: alkaloids, tannins, flavonoids, and anthraquinones. Alkaloids are the largest group of secondary plant substances and have antibacterial properties. The antibacterial mechanism of the alkaloids is thought to be by interfering with the peptidoglycan constituent components of bacterial cells, so that the cell wall layer is not fully formed and causes cell death [4].

Antimicrobial activity of tannin compounds by shrinking the cell membrane so that it interferes with the permeability of the cell membrane. As a result, cells cannot carry out living activities so that their growth is inhibited or even dies [4].

Phenols in the form of flavonoids and anthraquinones can denature protein bonds on the microbial cell membrane so that the cell membrane becomes lysed, this will facilitate the entry of compounds into the cell nucleus [25]. Flavonoids in Chinese ketepeng have antimicrobial, anti-inflammatory, antioxidant, anti-allergic effects and are effective for several groups of fungi or fungi [20]. The mechanism of flavonoids asAntibacterial agents are divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism [12]. In inhibiting the synthesis of nucleic acids, rings A and B of flavonoid compounds play an important role in the process of interclassification or hydrogen bonding, namely by accumulating nucleic acid

bases thereby inhibiting the formation of DNA and RNA.

The result of flavonoid interaction will also cause damage to cell wall permeability. In inhibiting the function of the cell membrane, flavonoids will form complex compounds from extracellular and dissolved proteins so that the cell membrane will be damaged and intracellular compounds will come out.

Meanwhile, in inhibiting energy metabolism by inhibiting the use of oxygen by bacteria, namely by preventing the formation of energy in the cytoplasmic membrane and inhibiting the motility of bacteria that play a role in antimicrobial activity and extracellular proteins [19].

CONCLUSION

Based on the results of research and discussion, it can be concluded that:

1. There was an effect of endophytic bacterial isolates from the leaves of the Chinese Ketepeng plant (Cassia alata L) on the growth of Staphylococcus aureus.
2. There are differences between treatments, namely the negative control treatment significantly different from BEKC 1, BEKC 2 and positive control, while positive control was significantly different from BEKC 1, BEKC 2 and negative control. Meanwhile, there was no significant difference between BEKC 1 and BEKC 2 . isolates

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