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MOLECULAR CHARACTERIZATION OF ENDOPHY MUSHROOMS AS ANTI-MICROBIAL FROM BLUME LEAVES (PREMNA PUBESCENS. BUAS-BUAS)

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ABSTRACT

Alternative utilization of natural resources through the use of endophytic microbes that live in every tissue in plants. Endophytic microbes can live in plant tissues in the form of bacteria or fungi. Endophytic fungi have the same ability as their host plants in producing secondary metabolite compounds. Plants that contain secondary metabolites and are known to be able to inhibit the growth of pathogenic bacteria are Blume (savage) plants.

Blume tree (*Premna pubescens*, Buas-buas) is a plant that contains antibacterial because it contains secondary metabolites and saponins, flavonoids including luteolin and apigenin which have a good effect on preventing bacterial growth. Endophytic fungi found in a plant do not all have the ability to inhibit bacterial growth. So, a study was conducted to determine which endophytic fungi have the ability as antibacterial. Furthermore, endophytic fungi will be identified molecularly to ascertain the types and species of fungi and bacteria that have potential as antibacterial.

The method in this research is to produce endophytic fungi that have the potential as antibacterial by first isolating the endophytic fungi present on Blume (buas-buas) leaves. All pure isolates of endophytic fungi found during the isolation process of Blume (buas-buas) leaves were tested for their antibacterial activity against the test bacteria. The pure isolates of endophytic fungi will then be identified molecularly by extracting DNA from the pure isolates of endophytic fungi which have the potential as antibacterial.

Based on the results of the tests carried out both on endophytic bacteria against pathogenic bacteria or endophytic fungi against pathogenic bacteria, the results obtained proved that Blume (savage) leaves contain substances such as flavonoids, alkaloids, phenolics, and saponins. Blume extract (buas-buas) is known to have antimicrobial ability to inhibit microbial growth in inhibiting the growth of pathogenic microbes.

Keywords: Isolation; Endophytic fungi; Blume (buas-buas) leaves; Molecular Identification.

I. INTRUDUCTION

Blume (savage) is one of the plants that are still not widely known and used by the people of Indonesia. This plant is used by the Malay community as a vegetable, specially included as a mixture in making spicy porridge. Blume (buas-buas) contains several secondary metabolites such as alkaloids, flavonoids, glycosides, triterpenoids, and saponins. Secondary metabolites are metabolic molecules that are produced from secondary metabolic processes in microorganisms where the results of these metabolisms are not the basic needs of a microorganism to live and grow.

Secondary metabolites in plants help plants to maintain a balanced system with the environment, to adapt according to environmental needs. The content of secondary metabolites found in Blume leaves (wild beasts), is also thought to be produced by a group of endophytic fungi that live on Blume leaves (savage). Endophytic fungi are widely explored as an alternative to bioactive compounds because of their ability to produce metabolites that have the potential to be developed into medicinal raw materials, 2009).

Endophytic fungi that can produce secondary metabolites according to their host plants, open up opportunities to produce secondary metabolites. If endophytic fungi isolated from a medicinal plant can produce alkaloids or secondary metabolites similar to the original plant or even in higher amounts, then we do not need to harvest the original plant to be taken as simplicial which will likely take decades to grow.

II. RESEARCH METHODS

The results of the isolation of Blume leaves (*Premna pubescens*, buas-buas), have endophytic fungi that have potential as antimicrobials. On young Blume leaves, 2 types of endophytic fungi, on medium Blume leaves, 4 types of endophytic fungi, and on old Blume leaves, 2 types of endophytic fungi.

Figure 1. Blume leaf (*Premna pubescens*, buas-buas)

Macroscopic Observation of Endophytic Fungal Colony Morphology

The results of macroscopic observations of endophytic fungal colony morphology that have been purified from samples of young Blume leaves, medium leaves, and old leaves found 2 endophytic fungal isolates from young leaves, the first young leaf endophytic fungal isolate (IJM1) and the second young leaf endophytic fungal isolate (IJM2), 4 isolates of medium leaf endophytic fungi, the first medium leaf endophytic fungus isolate (IJS1), the second medium leaf endophytic fungus isolate (IJS2), the third medium leaf endophytic fungal isolate (IJS3), and the fourth medium leaf endophytic fungal isolate (IJS4) and 2 isolates of endophytic fungi from old leaves, isolates of endophytic fungi from old leaves first (IJT1), isolates from endophytic fungi from old leaves (IJT2) are presented in Table 1.

Table 1. Results of Macroscopic Observations of Fungal Colonies

Endophytic Fungus Isolation		Microscopic Observation of Endophytic Fungus	
		Sporangium Shape	Sporangium Location
Young Leaves	I	Round	Edge
	II	Round	Edge
Medium Leaf	I	Round	Edge
	II	Round	Edge
	III	Ellipse	Edge
	IV	Round	Edge
Old Leaves	I	Round	Edge
	II	Round	Edge

Based on table 1 above, the results of observations, the first endophytic fungal isolate (IJM1), the first medium leaf endophytic fungal isolate (IJS1), and the first old leaf endophytic fungal isolate (IJT1) in each sample of young leaves, medium leaves, and old leaves have the same morphological characteristics, namely with a flat thickened colony shape, black color, regularly spread mycelium, coarse fibrous texture, and the colony grows upwards. The morphological characteristics of the second young leaf endophytic fungus (IJM2) colony were thickened flat colonies, white in color with turquoise edges, the mycelium was spread, the texture was like root fibers, and the colonies grew horizontally.

On medium leaves, the morphological characteristics of the second medium leaf endophytic fungal isolate (IJS2) were flat, thickened colonies, greenish-white in color, the mycelium grew spread, the texture was like rough velvet, and the colony growth was flat. The morphological characteristics of the third medium leaf endophytic

fungus (IJS3) colony were flat and thickened colonies, gray in color, regularly distributed mycelium, finely fibrous texture, and the colonies grew upwards. The morphological characteristics of the fourth medium leaf endophytic fungal isolate (IJS4) were flat and thickened colonies, white in color, the mycelium grew spread out, the texture was fine velvety and the colony growth was flat. The morphological characteristics of the second old leaf endophytic fungus isolate (IJT2), had a thin flat shape, turquoise green color, with diffuse mycelium, smooth velvety texture, and the colony grew horizontally.

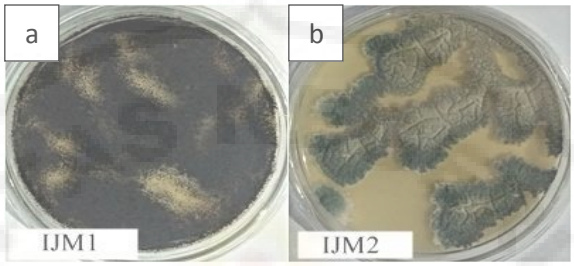

Figure 2. Macroscopic Isolate of Young Leaf Endophytic Fungus; (a) First Young Leaf Endophytic Fungus Isolate (IJM); (b) Second Young Leaves Endophytic Fungus Isolate (IJM2)

Based on the results of macroscopic observations of the morphology of endophytic fungal colonies that have been purified from samples of young leaves.

a. In the first young leaf endophytic fungal isolate (IJM1) the colony was thickened, black in color, had a regular spreading mycelium with a fibrous texture with the growth of endophytic fungal colonies upwards.

b. In the second young leaf endophytic fungal isolate (IJM2), the colony was flat and thickened, white in color with turquoise edges, had a diffuse mycelium with a texture like root fibers, and the colony growth was flat.

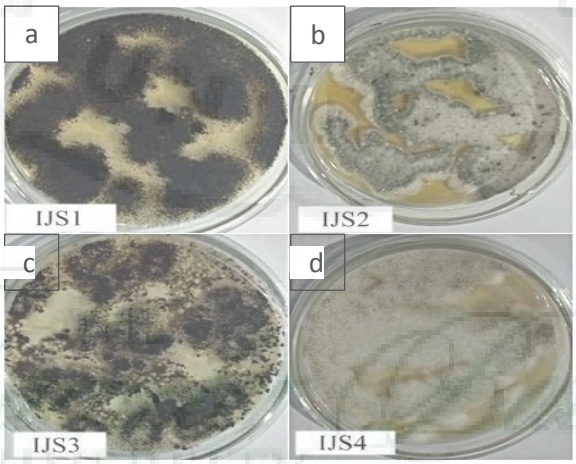

Figure 3. Macroscopic isolates of medium leaf endophytic fungi; (a) First Medium Leaf Endophytic Fungus Isolate (IJS1); (b) Second Medium Leaf Endophytic Fungus Isolate (IJS2); (c)Middle Leaf Endophytic Fungus Isolate (IJS3); (d) Fourth Medium Leaf Endophytic Fungus Isolate (IJS4).

Based on the results of macroscopic observations of endophytic fungal colony morphology that have been purified from samples of medium leaves.

a. In the first medium leaf endophytic fungal isolate (IJS1), the colony was flat, thickened, black in color, had a regular spreading mycelium with coarse fiber texture, and the growth of endophytic fungal colonies was upward.

b. In the second medium leaf endophytic fungal isolate (IJS2), the colony was flat, white in color, had a diffuse mycelium with a velvety texture, and the colony growth was flat.

c. In the third medium leaf endophytic fungal isolate (IJS3), the colony was thickened, gray in color, had a regular spreading mycelium with a fine fibrous texture with the growth of endophytic fungal colonies upwards.

d. In the fourth medium leaf endophytic fungal isolate (IJS4), the colony was thickened flat, white in color, had a diffuse mycelium, with a coarse fibrous texture, with the growth of flat endophytic fungal colonies.

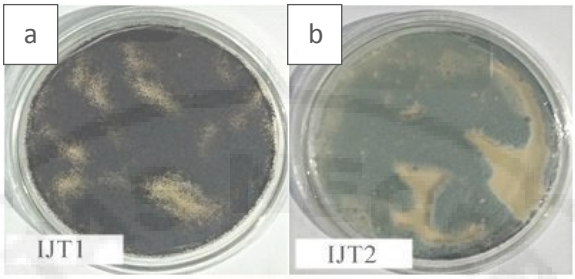

Figure 4. Macroscopic Isolate of Old Leaf Endophytic Fungus; (a) First Old Leaf Endophytic Fungus Isolate (IJT1); (b) Second Old Leaf Endophytic Fungus Isolate (IJT2).

Based on the results of macroscopic observations of the morphology of the endophytic fungal colonies that have been purified from samples of old leaves.

a. In the first old leaf endophytic fungal isolate (IJT1), the colony was flat, thickened, black in color, had a regular spreading mycelium with a coarse fibrous texture, the growth of the endophytic fungal colony was upward.

b. In the second old leaf endophytic fungal isolate (IJT2), the colony was thin flat, green in color, had a diffuse mycelium, with a smooth velvety texture, the growth of the endophytic fungal colony was flat.

RESULTS

Microscopic Observation of Endophytic Fungus

The results of microscopic observations of purified endophytic fungi from samples of young leaves, medium leaves, and old leaves found 2 isolates of endophytic fungi from young leaves, the first isolate of endophytic fungi from young leaves (IJM1), isolates of endophytic fungi from young leaves the second (IJM2), 4 isolates of fungi endophytic fungi from medium leaves, isolates of the first medium leaf endophytic fungi (IJS1), isolates of the second medium leaf endophytic fungi (IJS2), isolates of endophytic fungi of medium leaves (IJS3), and isolates of the fourth medium leaf endophytic fungi (IJS4). 2 isolates of endophytic fungi from old leaves, the first isolate of endophytic fungi from old leaves (IJT1), and isolates of endophytic fungi from old leaves to the second (IJT2) are presented in the table below. Characteristics were carried out through microscopic observations carried out by the slide culture method which were then observed using a microscope, with 10x and 20x magnifications.

Table 2. Observation Results of Microscopic Characterization of Endophytic Fungus

Endophytic Fungus Isolation		Microscopic Observation of Endophytic Fungus	
		Sporangium Shape	Sporangium Location
Young Leaves	I	Round	Edge
	II	Round	Edge
Medium Leaf	I	Round	Edge
	II	Round	Edge
	III	Ellipse	Edge
	IV	Round	Edge
Old Leaves	I	Round	Edge
	II	Round	Edge

Microscopic observations of endophytic fungal isolates can be seen from Figure 5 below: the results of observations of endophytic fungal isolates include the shape and location of the sporangium.

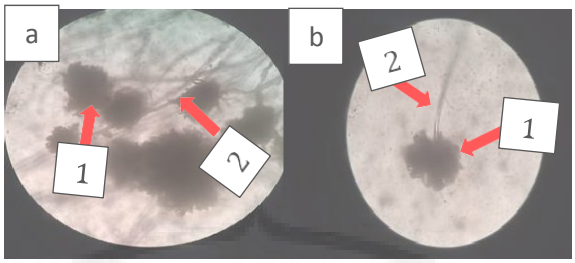

Figure 5. Microscopic Isolate of Young Leaf Endophytic Fungus; (a) First Young Leaf Endophytic Fungus Isolate (IJM1); (b) Second Young Leaf Endophytic Fungus Isolate (IJM2).

The results of microscopic observations of endophytic fungal isolates were obtained from the isolated leaves of Blume (*Premna pubescens*, buas-buas).

a. In the first fungal isolate of young leaves (IJM1) Figure 5, shows the sporangium and hyphae of the first young leaf endophytic fungal isolate, number 1 shows sporangium and number 2 shows hyphae. The first young leaf endophytic fungal isolate (IJM1) had a round sporangium, the sporangium on the first young leaf isolate was located at the tip of the hyphae.

b. In the second young leaf fungus isolate (IJM2).

The picture shows the sporangium and hyphae of the second young leaf endophytic fungal isolate, number 1 shows sporangium and number 2 shows hyphae. The second young leaf fungus isolate (IJM2) had a round sporangium, the sporangium on the second young leaf isolate was located at the tip of the hyphae

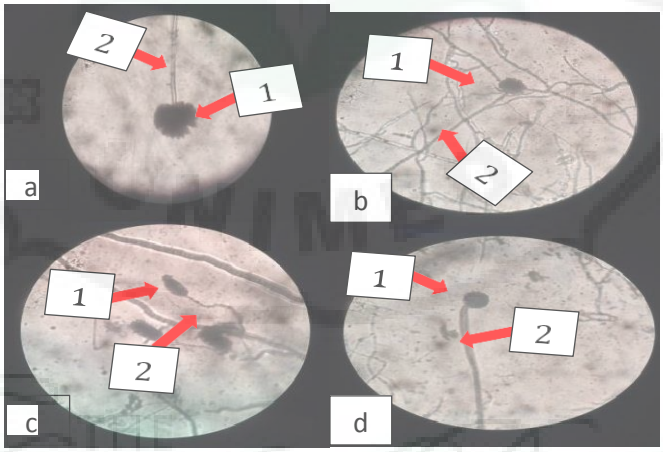

Figure 6. Microscopic isolates of medium leaf endophytic fungi; (a) First Medium Leaf Endophytic Fungus Isolate (IJS1); (b) Second Medium Leaf Endophytic Fungus Isolate (IJS2); (c) Middle Leaf Endophytic Fungus Isolate (IJS3); (d) Fourth Medium Leaf Endophytic Fungus Isolate (IJS4).

The results of microscopic observations of endophytic fungal isolates were obtained from the isolation of Blume (*Premna pubescens*, buas-buas) leaves.

a. In the first medium leaf fungus isolate (IJS1), the picture shows the sporangium and hyphae of the first medium leaf endophytic fungal isolate, number 1 shows sporangium and number 2 shows hyphae. The first medium leaf endophytic fungal isolate (IJS1) had a round sporangium, the sporangium on the first medium leaf isolate was located at the tip of the hyphae.

b. In the second medium leaf endophytic fungal isolate (IJS2) the image shows the sporangium and hyphae of the second medium leaf endophytic fungal isolate, number 1 is sporangium and number 2 shows hyphae. The second medium leaf endophytic fungal isolate (IJS2) had a round sporangium, the sporangium of the second medium leaf endophytic fungal isolate was located at the tip of the hyphae.

c. In the third medium leaf endophytic fungal isolate (IJS3), the image shows that the sporangium and hyphae of the third medium leaf endophytic fungal isolate (IJS3) have elliptical sporangium, the sporangium in the third medium leaf isolate is located at the tip of the hyphae.

d. In the fourth medium leaf endophytic fungal isolate (IJS4) the image shows the sporangium and the hyphae on the fourth medium leaf endophytic fungal isolate (IJS4) have round sporangium, the fourth medium leaf isolate sporangium is located at the tip of the hyphae.

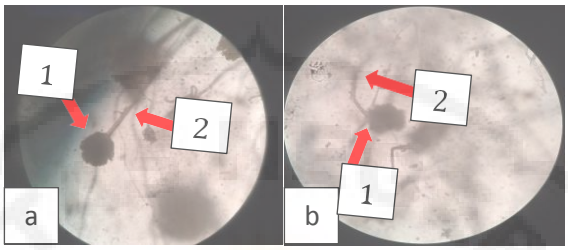

Figure 7. Microscopic Observation of Old Leaf Endophytic Fungus Isolates; (a) First Old Leaf Endophytic Fungus Isolate (IJT1); (b) Second Old Leaf Endophytic Fungus Isolate.

Based on the results of microscopic observations of endophytic fungal isolates obtained from the isolation of Blume (*Premna pubescens*, buas-buas) leaves.

a. In the old leaf endophytic fungal isolate (IJT1), the image shows the sporangium and hyphae of the first old leaf endophytic fungal isolate (IJT1) have rounded sporangium, the sporangium in the first old leaf isolate is located at the tip of the hyphae.

b. In the second old leaf endophytic fungal isolate (IJT2), the image shows the sporangium and hyphae of the second old leaf endophytic fungal isolate (IJT2) have round sporangium, the sporangium in the second old leaf isolate is located at the tip of the hyphae.

III. SELECTION OF ENDOPHYTIC FUNGUS AGAINST TEST BACTERIA

This test was carried out using six types of pathogenic bacteria, namely Bacillus subtilis ATCC 6051™, Enterococcus faecalis ATCC 29212™, Pseudomonas aeruginosa ATCC 27853™, Salmonella thypi ATCC 14028™, and Staphylococcus aureus ATCC 25923™. Streptococcus mutans ATCC 25175™. There were variations in the diameter of the inhibitory power formed in the six test bacteria. The results of the antibacterial test against the first test bacteria in the form of B. Subtillis ATCC 6051™ showed that the average diameter of the greatest inhibition was in the endophytic fungal isolate isolated from young leaves (IJM1) with an inhibitory value of 14.78 ± 2.58. In the medium leaf sample, (IJS1) showed a greater inhibitory value than the others, which was 16.87 ± 2.45. Meanwhile, in the old leaf sample, IJT2 showed a greater inhibitory value than IJT1 which was 10.63 ± 1.55.

In the second type of bacteria, E. faecalis ATCC 29212™ isolate showed the presence of inhibition formed in all endophytic fungal isolates. The diameter of the greatest inhibition was in the endophytic fungal isolate isolated on old leaves (IJT1) with an inhibitory value of 14.73 ± 1.31. Endophytic fungal isolates on young leaves (IJM1) also showed a greater inhibitory value than IJM2, which was 12.37 ± 1.79. Meanwhile, medium leaf (IJS4) had a greater inhibitory value than the other medium leaf endophytic fungi isolates, which was 11.30 ± 2.74.

The third test bacterial isolate, P. aeruginosa ATCC 27853™, showed the presence of inhibition formed in all endophytic fungal isolates. The diameter of the greatest inhibition was found in the first endophytic fungal isolate on old leaves (IJT1), with an inhibitory value of 16.15 ± 2.41. In young leaves, the highest inhibitory value was at IJM2 of 11.92 ± 1.11. As for medium leaves, the greatest inhibitory value was IJS1 with a value of 13.27 ± 0.55.

The fourth test bacterial isolate, S.thymi ATCC 14028™, showed the highest inhibitory value of the first endophytic fungal isolate on old leaves (IJT1) with a value of 14.80 ± 1.61. In young leaves, the second isolate of endophytic fungi (IJM2) was an isolate with a higher inhibitory value than the first isolate (IJM1), which was 10.33 ± 1.18. On medium leaves, the third isolate (IJS3) was the isolate that had a higher inhibitory value than the other isolates, which was 11.10 ± 6.12.

The fifth test bacterial isolate, in the form of S. aureus ATCC 25923™, showed the highest inhibitory value of the first endophytic fungal isolate on old leaves (IJT1) with a value of 14.45 ± 3.35. In young leaves, the first isolate of endophytic fungus (IJM1) was also superior again having a greater inhibitory value than the second isolate (IJM2), which was 13.57 ± 0.66. In medium leaves, the first isolate (IJS1) also had a greater value than the other medium leaf isolates, which was 13.30 ± 1.11.

The sixth test bacterial isolate, S.mutans ATCC 25175™, showed that the average diameter of the greatest inhibition was in the endophytic fungal isolate isolated on medium leaves (IJS3) with an inhibitory value of 17.60 ± 2.99. In the sample of young leaves, IJM2 showed a greater inhibitory value than the others, which was 15.77 ± 2.36. Meanwhile, in the old leaf sample, IJT2 showed a greater inhibitory value than IJT1 which was 13.47 ± 4.03.

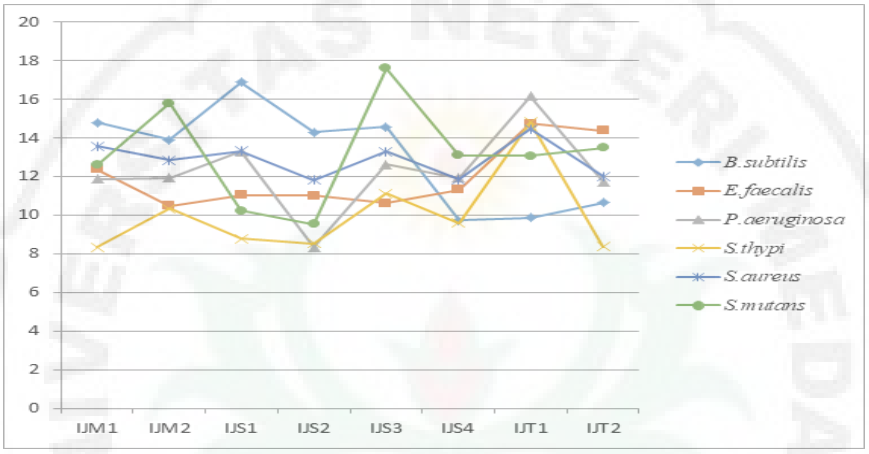

Figure 8. Graph of Average Selection of Endophytic Fungus against Test Bacteria

Based on Figure 8 above, it can be seen that the highest inhibitory value of the old leaf endophytic fungus isolates on the first old leaf (IJT1) against the test bacterium Pseudomonas aeruginosa with a value of 16.15 ± 2.4. On medium leaves, the third isolate (IJS3) had the highest inhibitory value against the test bacteria Streptococcus mutants with a value of 17.60 ± 2.99 compared to the other three isolates from all the tested bacteria. As for the young leaves, the first isolate (IJM1) had the highest inhibitory value against the test bacteria Bacillus subtilis with a value of 14.78 ± 2.58 compared to IJM2.

When viewed from each endophytic fungal isolate, the first young leaf isolate (IJM1) had a superior inhibitory value than IJM2 for the three types of bacteria tested (Bacillus subtilis, Enterococcus faecalis, and Staphylococcus aureus), while the second young leaf fungus isolate (IJM2) was superior to the first young leaf fungus (IJM1) against the three types of bacteria tested (Pseudomonas aeruginosa, Salmonella thyoi, and Streptococcus mutants.

In medium leaves, the first isolate had superior ability compared to other medium leaf isolates against test bacteria (Bacillus subtilis, Enterococcus faecalis, Pseudomonas aeruginosa, and Staphylococcus aureus. While the third young leaf isolate (IJS3) had superior inhibitory values against the two types of test bacteria. (Salmonella thyroid and Streptococcus mutans) compared to other medium leaf endophytic fungi isolates. In old leaves, the first endophytic fungal isolate (IJT1) had a superior inhibitory value compared to IJT2 against several test bacteria (Enterococcus faecalis, Pseudomonas aeruginous, Salmonella type, and Staphylococcus aureus), while the second old leaf isolate (IJT2) had superior inhibition against the test bacteria (Bacillus subtilis, and Streptococcus mutans).

IV. RESULTS OF DNA AMPLIFICATION OF ENDOPHYTIC FUNGUS ISOLATES POTENTIALLY AS ANTIBACTERIAL

Bacterial isolates of potential endophytic fungi isolates, namely (IJM1), (IJM2), (IJS1), (IJT1), and (IJT2), were then extracted and amplified using a polymerase chain reaction (PCR) machine. The results of the amplification with the ITS rDNA marker are as follows:

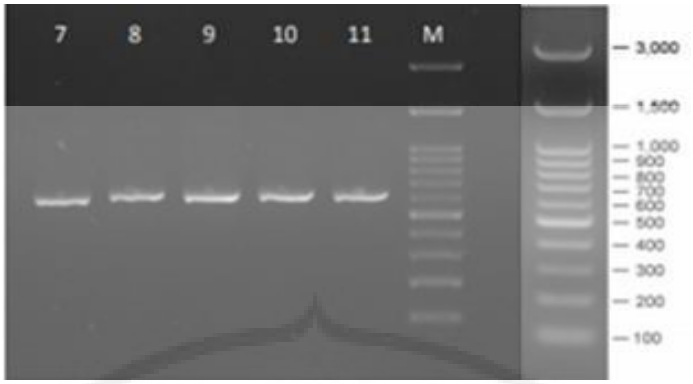

Figure 9. Results of Electrophoresis Visualization with ITS rDNA (IJM1), (IJM2), (IJS1), (IJT1), and (IJT2).

Figure 8 above shows the results of electrophoresis visualization explaining the results of DNA amplification of endophytic fungal isolates from Blume (savage) leaves which produced 5 bands, namely a specific band measuring ±600bp and a thick band of five endophytic fungal isolates. This shows that the ITS regions of five isolates were successfully amplified using universal primer pairs ITS 1 and ITS 4 with the right PCR conditions and sufficient concentration of DNA templates and optimal annealing temperatures.

Isolate of Blume Leaf Endophytic Fungus (Buas-buas) Potentially as Antibacterial

The DNA that has been successfully extracted and visualized by electrophoresis is then followed by a sequencing process. The results of the sequencing of the five bacterial isolates are second (IJM2), the first medium leaf fungus isolate (IJS1), the first old leaf fungus isolate (IJT1), and the second old leaf fungus isolate (IJT2). at PT. Genetic Science Indonesia found that:

a. IJM1 isolate was 877 bp (base pairs),

b. IJM2 isolates amounted to 1130 bp (base pairs),

c. IJS1 isolates amounted to 924 bp (base pairs),

d. IJT1 isolates amounted to 1039 bp (base pairs),

e. IJT2 isolates amounted to 1048 bp (base pairs).

The number of nucleotides obtained was then carried out by homology analysis to determine the name of the endophytic fungal isolate. The DNA sequencing of endophytic fungal isolates was analyzed using the Basic Local Alignment Search Tool (BLAST). The analysis of the BLAST results provides information and verifies what fungi have similarities to the sample DNA sequences so that they can be used to identify endophytic fungal isolates. The description is the title of information which usually consists of genus, species, type of strain, type of gene/DNA fragment, and type of complete DNA sequence displayed per such information (partial sequence means only part of the DNA sequence of the actual total gene/fragment, while full CDS is complete gene coding DNA sequence).

Max Score is the total value obtained from the alignment between the input sequence/query with the database sequence. This value is obtained from the calculation of the matrix, which is a substitution matrix. Total Score is the total score obtained from all alignments between input sequences (queries) and parallel database sequences. This value can be different from the max score if there is more than one alignment that occurs. Query Coverage is a percentage that describes how big/long the fit of the input sequence is when compared to the target DNA sequence. If the input DNA sequence covers all the target DNA sequences in the NCBI database, then the percentage is 100%. Percentage Identity is a percentage that shows how well the data match the input DNA sequence with the target DNA sequence (Stackerbrandt and Goebel, 1994). The results of the BLAST analysis of endophytic fungal isolates are as follows:

a. That IJM1 isolate has homology (percentage identity) 99.64% with Aspergillus aculeatus CBS 172.66 with 100% query cover, max score 1014, total score 1614. Aspergillus uvarum with query cover 99%, max score 1014, total score 1616, and percentage identity 96.64%. Aspergillus japonicus CBS 114.51 has

a query cover of 100%, a max score of 1009, a total score of 1610, and a percentage identity of 99.64%. Aspergillus assiutensis AUMC 5748, query cover 99%, max score 1003, total score 1608, and percentage identity 99.64%. Aspergillus brunneoviolaceus NRRL 4912, has a query cover of 95%, a max score of 968, a total score of 1544, and a percentage identity of 99.64%. Aspergillus homomorphus CBS 101889, has a query cover of 100%, a max score of 876, a total score of 1442, and a percentage identity of 99.64%. Aspergillus aculeatinus CBS 121060, has a query cover of 82%, a max score of 817, a total score of 1344 and a percentage identity of 99.64%. Aspergillus saccharolyticus, has a query cover of 100%, a max score of 715, a total score of 1136, and a percentage identity of 99.64%. Aspergillus heteromorphus CBS 117.55, has a query cover of 100%, a max score of 676, a total score of 1137, and a percentage identity of 99.64%. Penicillium costaricense CBS 140998, has a query cover of 100%, a max score of 669, a total score of 1069, and a percentage identity of 99.64%. According to Hagstrom (2002), if the similarity is 93%, it is considered the same species and according to Schloss and Handelsman (2004), if the maximum similarity is 97%, it is considered the same species. So, to determine IJM1 isolates, a phylogenetic analysis was carried out to see which fungi were closer to the first young leaf endophytic fungal isolates (IJM1).

b. The results found that the second young leaf endophytic fungus isolate (IJM2) had a query cover of 99%, a max score of 1057, a total score of 2053, and a percentage identity of 98.49% with Aspergillus flavus ATCC 16883 According to Hagstrom (2002), if the similarity is 93%, it is considered the same species and according to Schloss and Handelsman (2004), if the maximum similarity 97% is considered one species. Then the possibility of the second young leaf fungus isolate (IJM2) with a probability >97% is Aspergillus flavus ATCC 16883 which then determines the relationship phylogenetic analysis is carried out to see which fungal species are related to the second young leaf endophytic fungal isolate (IJM2).

c. Based on the results, it was found that the first medium leaf endophytic fungus isolate (IJS1) had a query cover of 100%, a max score of 1101, a total score of 1680, and a percentage identity of 99.83% with the fungus Aspergillus fumigatus ATCC 1022. According to Hagstrom (2002), if the similarity is 93% then considered the same species, and according to Schloss and Handelsman (2004), if the maximum similarity is 97% is considered one species. So, the possibility of IJS1 isolates with probability > 97% is Aspergillus fumigatus ATCC 1022 then to determine the relationship phylogenetic analysis is carried out to see fungal species that are related to the first medium leaf endophytic fungal isolate (IJS1).

d. The results found that the first old leaf endophytic fungus isolate (IJT1) had a query cover of 100%, a max score of 1088, a total score of 1902, and a percentage identity of 99.66% with Aspergillus flavus ATCC 16883. According to Hagstrom (2002), if the similarity is 93%, then considered the same species and according to Schloss and Handelsman (2004), if the maximum similarity 97% is considered one species. So, the possibility of IJT1 isolates with probability > 97% is Aspergillus flavus ATCC 16883 which then to determine the relationship phylogenetic analysis was carried out to see fungal species that have a relationship with endophytic fungal isolates IJT1.

e. Based on the results, it was found that IJT2 isolates had a query cover of 100%, a max score of 1083, a total score of 1909, and a percentage identity of 99.50% with Aspergillus flavus ATCC 16883. According to Hagstrom (2002), if the similarity is 93%, it is considered the same species, described by Schloss and Handelsman (2004), if the maximum similarity is 97% is considered one species. So, the possibility of IJT2 isolates with probability >97% is Aspergillus flavus ATCC 16883 which then determines the relationship phylogenetic analysis is carried out to see fungal species that are related to IJT2 endophytic fungal isolates.

V. CONCLUSION

Based on the results of the research conducted, it can be concluded that in research to produce endophytic fungi and bacteria with antibacterial capabilities, first isolate the endophytic fungi present on each Blume leaf (buas-buas). All fungi and bacteria that live on the leaves of Blume (buas-buas) were tested for their antibacterial activity against the test bacteria, namely Escherichia coli and Staphylococcus aureus.

Fungi and bacteria that have shown antibacterial ability will be identified molecularly. Based on the results of the tests carried out both on endophytic bacteria against pathogenic bacteria or endophytic fungi against pathogenic

bacteria, the results obtained proved that Blume (savage) leaves contain substances such as flavonoids, alkaloids, phenolics, and saponins. Blume extract (buas-buas) is known to have antimicrobial ability to inhibit microbial growth in inhibiting the growth of pathogenic microbes.

Blume leaves (buas-buas) contain secondary metabolites such as alkaloids, flavonoids, glycosides, triterpenoids, and saponins. The content of secondary metabolites found in Blume leaves (Buas-buas), is also thought to be produced by groups of endophytic fungi and endophytic bacteria that live on Blume leaves (Buas-buas). Microscopic observation of endophytic bacteria with gram staining at 40x magnification under a microscope showed that endophytic bacteria isolates were gram-negative bacteria in the form of bacilli (rods) arranged like elongated chains. The results of the biochemical test of endophytic bacterial isolates showed that they were from the Enterobacteriaceae family with the types of endophytic bacterial species including Enterobacter aerogenes, Shigella sonnei, Klebsiella pneumoniae, Enterobacter cloacae, Salmonella arizonae, Enterobacter agglomerans. On microscopic observation found several types of endophytic fungal species including Aspergillus sp. Fusarium sp. And Curvularia sp.

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