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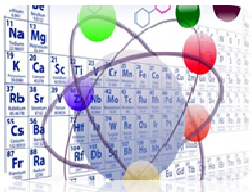

Antibacterial Activity of Endophyte Fungus from Sambiloto Flowers (*Andrographis paniculata*) on Black Rice Growing Media

Rizka Purwanti[1*], Iryani Iryani[2], Riga Riga[3] dan Mariam Ulfah[4]

[1,2,3] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Padang State University, Prof. Dr. Hamka road, West Freshwater, Padang City, 25132, Indonesia.

[4] Cirebon Muhammadiyah College of Health Sciences, Kalitanjung road, Harjamukti, Cirebon City, 45143, Indonesia.

* Corresponding author : riskapurwanti018@gmail.com

ABSTRACT

Sambiloto plant (A. paniculata) is widely reported to have secondary metabolites that act as antibacterial. The potential is not only derived from the natural A. paniculata plant but can also be explored from its endophytic fungus. This study aimed to determine the antibacterial activity of ethyl acetate extract of endophytic fungus BS isolated from bitter flower (A. paniculata) on black rice growing media against Staphylococcus aureus, Streptococcus mutans, and Pseudomonas aeruginosa bacteria. The antibacterial activity test of the extract was carried out by disc diffusion method. The results obtained showed that the ethyl acetate extract of BS endophytic fungus grown on black rice media showed activity to inhibit the growth of Staphylococcus aureus, Streptococcus mutans, and Pseudomonas aeruginosa bacteria at concentrations of 10%, 30%, and 50%. In conclusion, the ethyl acetate extract of the endophytic mushroom BS isolated from the bitter flower (A. paniculata) can be used as an antibacterial.

Keywords: Antibacterial, Endophytic Fungus, Sambiloto Flower, Black Rice

1. INTRODUCTION

Infectious diseases are one of the diseases that cause high mortality rates, especially in Indonesia developing countries like Indonesia. In Indonesia, infectious diseases are included in the category of the ten most common causes of death.[1] Bacteria are the main cause of infectious diseases. Antibiotics are often used to treat infectious diseases caused by bacteria, excessive and irrational use of antibiotics can cause problems and become a threat to the world of health, especially the occurrence of bacterial resistance to antibiotics.[2] Cases of antibiotic resistance have recently emerged rapidly along with the use of antibiotics as a cure for infectious diseases. This situation encourages the importance of efforts to find new antibiotic compounds that are natural, easy to develop at low cost, and available continuously, one of which is new bioactive

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compounds derived from natural ingredients. The most widely used natural wealth for the discovery of new drugs is derived from medicinal plants.[3] One of the medicinal plants that has been reported to produce secondary metabolites with antibacterial activity is Sambiloto Plant (A. paniculate). According to the Food and Drug Administration (BPOM), this plant is included in the list of superior plants that can be developed in the pharmaceutical industry, because it contains secondary metabolites that are very useful.[4] Previous phytochemical studies reported that bitter plants contain a variety of secondary metabolites such as saponins, flavonoids, alkaloids, terpenoids, steroids, and tannins.[5]

The potency of medicinal plants is also related to the microorganisms that live in the plant tissue. These microorganisms are known as endophytic microbes, one of which is endophytic fungi. Endophytic fungi are fungi that live in plant tissues at a certain time and have the ability to form a certain colony without harming the host plant.[6] The ability of endophytic fungi to produce the same bioactive compounds as their host plants provides an opportunity to obtain natural, inexpensive, and environmentally friendly sources of medicinal ingredients. Research on biological studies of endophytic fungi isolated from roots, stems, twigs, and leaves of bitter gourd (A. paniculata) has been previously reported. A recent study reported that endophytic fungi were also isolated from sambiloto flowers grown on white rice media with mushroom code BS. This endophyte fungus has antibacterial activity and can produce several secondary metabolites such as terpenoids, alkaloids, and phenolics.[7] In addition to the host plant, the mushroom growing media also affects the secondary metabolites produced.

In this study, researchers grew BS endophytic fungi from bitter flowers on black rice. Black rice is one type of rice consumed by the people of Indonesia. Secondary metabolites found in black rice include flavonoids, tannins, alkaloids, and steroids.[8] The reddish black color of black rice is caused by anthocyanins contained in the epidermal cells. The body uses these anthocyanins as antioxidants, anti-inflammatory agents, tumor cell inhibitors, and diabetes and obesity prevention agents.[9] This study of the antibacterial activity of the BS fungus from the bitter flower (Andrographis paniculate) on black rice growing media was the first to be conducted.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The materials used in this study were bitter plants (A. paniculata), ethyl acetate, 70% ethanol, 3.5% NaOCl, aquades, black rice, Potato Dextrose Agar (PDA), DMSO, Mueller Hinton Agar (MHA), paper discs, amoxicillin, and three test bacteria namely Staphylococcus aureus, Streptococcus mutans, and Pseudomonas aeruginosa. The tools used are incubator, petri dish, Laminar Air Flow (LAF), Erlenmeyer, skewer, autoclave, measuring cup, beaker, stirring rod, separating funnel, ose needle, analytical balance, digital balance, caliper, rotary evaporator, tweezers, filter paper, and handscon.

2.2. Research Procedure

2.2.1 Endophytic Fungus Inoculation

The bitter plant (A. paniculate) was obtained from the Dadok area, Tunggul Hitam, Koto Tangah District, Padang City. Sambiloto flowers measuring 2x2 cm are first washed with running water to remove dirt on the surface of the flowers. Then sterilized by immersing it in 70% alcohol solution for 45 seconds and 3.5% NaOCl solution for 30 seconds and followed by rinsing using distilled water. After that, as a negative control, sterile sambiloto (A. paniculate) flowers were attached to PDA solid media. Then the size of 1 x 1 flower was cut to be inoculated on PDA solid media and incubated at 28^0C for 5-7 days. To obtain a single isolate, the endophytic fungi that had grown were transferred to other PDA media.[10]

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2.2.2 Cultivation and Manufacturing of Endophytic Fungus Extracts

BS endophytic fungus isolates were transferred with a size of 1x1 cm from solid media into natural growth media, namely black rice media that had been autoclaved at 121^0C and 15 lbs pressure as much as 25 Erlenmeyer. Endophytic fungus BS from the bitter flower (A. paniculata) was cultivated on a large scale according to its optimum cultivation time of 21 days. The cultivated mushrooms were extracted 3 times by maceration method using 50 ml of ethyl acetate solvent for 3x24 hours. Then the extraction results obtained were filtered using filter paper, and the filtrate from the filtration was concentrated using a rotary evaporator to obtain a concentrated extract which would then be tested for its antibacterial activity and secondary metabolite content.[7]

2.2.3 Secondary Metabolite Content Test

Steroids and Terpenoids

Extract of BS Endophytic Mushroom was put into a test tube, then add chloroform ammonia and H_2SO_4 2N, and shake vigorously. Let stand the mixture until it forms two layers, where the top layer is acid and the bottom layer is chloroform. the bottom layer is taken and placed on the drip plate. After the chloroform evaporates, add H_2SO_4 p.a and anhydrous acetic acid. Positive samples containing terpenoids were indicated by the formation of a red color and the formation of a green-blue color indicating that they were positive for steroids.

Alkaloids

The top layer or acid layer in the steroid and terpenoid test was transferred with a dropper into a test tube and then Dragendorff, Wagner, and Mayer reagents were added. The results were declared positive for containing alkaloids if there was a change in color to orange after the addition of Dragendorff's reagent, brown after the addition of Wagner's reagent, and white after the addition of Mayer's reagent.

Phenolic

The concentrated extract of BS endophytic fungus that has been obtained is put on a drip plate, then add 1% $FeCl_3$ solution. If a blue-black color is formed, it indicates a positive sample containing phenolics.[11]

2.2.4 Antibacterial Activity Test

The antibacterial activity of BS endophytic mushroom extract was tested on Staphylococcus aureus, Streptococcus mutans, and Pseudomonas aeruginosa bacteria using the disc diffusion method. 10 µL of endophytic mushroom extract BS (10%, 30%, and 50% concentration) was added to each test bacteria inoculant, a positive control (amoxicillin), and a negative control (solvent). After that, it was incubated for 24 hours at 37°C. The presence of a clear zone around the paper disc was used to determine the potential antibacterial activity of the extract. The diameter of the clear zone was measured which was expressed in the inhibition zone. The antibacterial activity test was carried a triple. The test process was carried out under aseptic conditions.[12]

3. RESULTS AND DISCUSSION

The results showed that the endophytic fungus obtained from the isolation of the flower of the bitter plant (A. paniculata) had morphological characteristics, namely white colonies with a rounded, rounded shape. The structure of the BS fungal colonies was slightly rough with a thin uniform distribution over the entire surface of the media. The lower surface of the BS endophytic fungus is also in the form of white colonies as shown below:

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Figure 1. Endophytic fungi morphology BS

BS endophytic fungus isolates were cultivated in natural media, namely 25 Erlenmeyer containing black rice media (black rice), black rice was chosen because it contains carbohydrates that can be utilized by fungi as a source of nutrition and also has a variety of secondary metabolites that may be attracted by fungi. endophyte. This cultivation aims to produce a large amount of extract mass according to the optimum time for fungal growth that has reached the stationary phase. The stationary phase is when the growth and death rates of microbes are the same, so that the total number of microbes produced will remain constant.[13] The results of previous studies proved that the optimum cultivation time of BS mushrooms in producing large amounts of secondary metabolites was in the third week.[7]

Endophytic fungi were cultivated on black rice growing media for three weeks which were then extracted using ethyl acetate solvent by maceration method for 3 x 24 hours. The ethyl acetate filtrate of endophytic mushroom BS obtained was as much as 3 liters and after being evaporated it produced 4.76 grams of concentrated extract. The concentrated ethyl acetate extract was then tested for secondary metabolites to determine the bioactive compounds contained in the sample.[14]

Table 1. Secondary metabolite content test results

No.	Chemical Compound	Reactor	Test results	Information
1	Alkaloids	Dragendorf	+	An orange precipitate is formed
		Wagner	+	Chocolate precipitate formed
		Mayer	-	No white precipitate is formed
2.	Terpenoids	$CHCl_3$ and H_2SO_4	+	Formed in red
3.	Steroids	$CHCl_3$ and H_2SO_4	-	No green-blue color formed
4.	Phenolic	$FeCl_3$	+	Formed in blue-black

The results showed that the ethyl acetate extract of endophytic fungi BS positive contained alkaloids, terpenoids, and phenolic compounds. This compound is a compound that acts as an antibacterial that can inhibit the growth of bacteria. Alkaloids have the ability to kill bacteria because they can prevent the formation of peptidoglycan in bacterial cells so that the cell wall layer is not completely formed and causes cells to die.[15] Alkaloids also prevent protein synthesis which makes them able to interfere with bacterial metabolism. Alkaloid compounds can inhibit the growth of gram-negative and gram-positive bacteria.[16]

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Terpenoids can stop bacterial growth, because they react with transmembrane proteins on the outer membrane of the bacterial cell wall, forming strong polymeric bonds that can cause damage. Damage to transmembrane proteins as the entrance and exit of compounds will reduce the permeability of the bacterial cell wall which will result in bacterial cells lacking nutrients and that it can stop bacterial growth and cause death[17]. Phenolic compounds can act as antibacterials by interacting with bacterial cells through an absorption process involving hydrogen bonds, disrupting the work of the cytoplasmic membrane, active transport, and proton strength.[18]

Testing the antibacterial activity of ethyl acetate extract of endophytic fungus BS was carried out by the paper disc diffusion method. This method was chosen because the procedure is easy, simple, and often used to determine antibacterial activity because it can determine the diameter of the inhibition zone directly.[19] The bacteria used were Staphylococcus aureus, Streptococcus mutans, and Pseudomonas aeruginosa, with the extract concentration, used consisting of three variations, namely 10%, 30%, and 50%. This antibacterial activity was carried out three times and the test results were expressed as zones of inhibition which are shown in the following table:

Table 2. Data on the diameter of the inhibition zone of BS endophytic fungus extract against test bacteria

Concentration	Test bacteria		
	S.aureus	***S. mutans***	***P. aeruginosa***
10%	7,06±0,92	8,23±0,36	5,77±0,26
30%	8,85±0,64	10,79±0,70	8,32±0,80
50%	10,50±0,94	12,40±0,64	11,07±0,66
Positive Control (+)	13,15±0,76	15,96±0,66	15,82±0,75

Based on the data above, it was proven that the ethyl acetate extract of the endophytic fungus BS gave an inhibitory power to the test bacteria. The diameter of the inhibition zone showed that the inhibition of bacteria was getting stronger with increasing extract concentration, this was due to an increase in the concentration of bioactive substances which caused higher antibacterial activity. In Table 2. it can be seen that the extract concentrations of 10% and 30% have moderate antibacterial activity, but at a concentration of 50% the antibacterial activity is included in the strong category, this is for the level of antibacterial activity which is divided into 4 levels, namely weak, moderate. , strong, and very strong. Bacterial activity is said to be weak if the inhibition zone has a clear diameter of <5 mm, the medium category is between 5-10 mm, the strong category is between 10-20 mm, and very strong if the clear zone diameter is > 20 mm.[20]

Differences in sample inhibition were also seen in each of the tested bacteria, this was due to differences in the composition of the cell walls of each bacterium. Bacterial cell walls are composed of peptidoglycan which will determine the shape of the cell later. Peptidoglycan is found in both gram-positive and gram-negative bacteria, but with a slightly different structure. Gram-positive bacteria have a cell wall composed of layers of thicker peptidoglycan, while gram-negative bacteria have a thinner peptidoglycan layer so that the walls of gram-positive bacteria are more easily damaged and produce a large inhibition zone.[21] In this study, Staphylococcus aureus and Streptococcus mutans were gram-positive bacteria, while Pseudomonas aeruginosa had gram-negative cell walls so that the diameter of the inhibition zone was larger for Staphylococcus aureus and Streptococcus mutans compared to Pseudomonas aeruginosa.

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4. CONCLUSION

Based on the results of this study, it can be concluded that the ethyl acetate extract of BS endophytic fungus showed the ability to inhibit the growth of the test bacteria with the smallest inhibition zone shown in the gram-negative bacterium Pseudomonas aeruginosa.

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REFERENCES

1. Ministry of Health of the Republic of Indonesia. Results of Basic Health Research 2018. Indonesian Ministry of Health, 53(9), 1689–1699.
2. Yulia, R., Mariza, J. W., & Herawati, F. **(2020).** Bacterial Profile and Antibiotic Use in Pneumonia Patients at Dr. Soetomo General Hospital. 21–27
3. Burhamzah, R., & Rante, H. **(2020).** Isolation and Screening of Marine Omycetes Produce Antibacterial Compounds of Multi Drug Resistance from Galesong Beach Marine Sediments. Pharmacy and Pharmacology Magazine. 23(3), 79–81
4. Royani, J. I., Hardianto, D., & Wahyuni, S. **(2014).** Analysis of Andrographolide Content in Sambiloto Plants (Andrographis paniculata) from 12 locations in Java. Indonesian Journal of Biotechnology & Bioscience,1(1), 15-0.
5. Nugroho, E., Rahardiningtyas, D., B. Wicaksono Putro., & R. Wianto. **(2016).** The Effect of Sambiloto Leaf Extract (Andrographis paniculata Ness.) on the Killing Power of Leptospira sp. Health Research and Development Media, vol. 26, no. 2, 77–84.
6. Murdiyah, S. **(2017).** Endophytic Fungi in Various Medicinal Plants in Evergreen Forest Areas in Baluran National Park and Development Potential as Practical Instructions for Mycology Courses. J Indonesian Biol Educator. 3(1), 2
7. Suhanah, R., Suryelita, S., Ethics, S. B., Ulfah, M., & Riga, R. **(2021).** Endophytic Fungus Isolated from Andrographis Paniculata (Sambiloto) Flower as a Source of Antibacterial Compounds. Journal of Indonesian Pharmaceutical People, 4(1), 139-48.
8. Agus, K. A. **(2016).** Identification of Anthocyanin Compounds and Secondary Metabolites from Ethanol Extract of Black Glutinous Rice (Oryza Sativa. L) in Its Use as an Alternative Treatment of Dengue Hemorrhagic Fever. Medicamento Scientific Journal. Vol. 2, No.1.
9. Nailufar, A. A., Basito & Anam, A. **(2012).** Study of Characteristics of Black Sticky Rice (Oryza sativa glutinosa) on Several Types of Packaging During Storage. Journal of Food Technology. Vol. 2. No.1, 122-132.
10. Riga, R., & Euis Holisotan H. **(2021).** Cytotoxic and Antibacterial Activity of Ethyl Acetate Extract of Endophytic Fungus Colletotrichum Gloeosporioides. Udayana Journal of Pharmacy 10(2), 193
11. Elita A., Saryono S., & Christine J. **(2013).** Determination of Optimum Time for Antimicrobial Production and Phytochemical Test of Fermented Crude Extracts of Endophytic Bacteria Pseudomonas sp. from Dahlia Plant Bulbs (Dahlia variabilis). J. Ind. Che. Acta. Vol. 3, No.2, Pg. 56-62
12. Riga, R., Nizar H., Armin Q., Chantale Z., Oliver K., & Euis Holisotan H. **(2019).** Secondary Metabolites from Diaporthe Lithocarpus isolated from Artocarpus Heterophyllus. Natural Product Research 35(14), 2324–28
13. Iqlima, D., Ardiningsih, P., & Wibowo, M. A. **(2017).** Antibacterial Activity of B2D Endophytic Bacteria Isolate From Yakon (Smallanthus Sonchifolius) Stem, Staphylococcus Aureus And Salmonella Typimurium. Jkk, 7(1), 36–4.
14. Yanti, S., & Vera, Y. **(2019).** Phytochemical screening of wuluh starfruit leaf extract (Averrhoa bilimbi). Indonesian Health Scientific Journal, 4(2), 41–46.
15. Pertiwi, F. D., Rezaldi, F., & Puspitasari, R. **(2022).** Test of Antibacterial Activity of Telang Flower (Clitoria ternatea L.) Ethanol Extract Against Staphylococcus epidermidis Bacteria. Bioscience-Tropic (Bioscience-Tropic), 7(2), 57–68.

e-ISSN : 2622-4968, p-ISSN : 2622-1349

16. Compean, K.L., & Ynalvez R.A. **(2014).** Antimicrobial Activity of Plant Secondary Metabolites. *A Review, Reserach of Medical Plant*. pp. 1-10.
17.Cowan, M. **(1999).** Plant Product as Antimicrobial Agent. *Clinical Microbiology Reviews, 12(4).* 564-582.
18. Harbor. **(1987).** Phytochemical Methods, A Guide to Modern Ways of Analyzing Plants. Translation; K. Padmawinata and I. Sudiro. Bandung : Bandung Institute of Technology.
19. Ogioni. **(2015).** Significant Differences Characterize The Correlation Coefficients Between Biocide And Antibiotic Susceptibility Prifiles In Streptococcus aureus. *Curr Pharm 12, no. 16*, 25-47.
20. Kumowal S., Fatimawali F., & Jayanto I. **(2019).** Antibacterial Activity Test of White Galangal (Alpinia galanga (L.) Willd) Extract Against Klebsiella pneumoniae Bacteria. PHARMACON. 8(4), 781-790.
21. Poetry A., Fatimawali., & Paulina V. Y. **(2019).** Inhibitory Test of Red Galangal Rhizome Extract(Alpiniapurpurata K.Schum) Against Bacterial Growth Klebsiella pneumoniae Isolate Sputum in Patients with Ceftriaxone Antibiotic-Resistant Pneumonia. Pharmacon 8:(1), 11-21.

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