Walisongo Journal of Chemistry

Walisongo Journal of Chemistry Vol. 6 Issue 2 (2023), 97-103
ISSN: 2621-5985 (online); 2549-385X (print)
DOI: https://doi.org/10.21580/wjc.v6i1.13241

Antibacterial Effectiveness of BS Endophyte Mushroom Extract on Media Growing Red Rice

Iryani Iryani[1*], Radia Sagita Pramesti[1], Riga Riga[1], Mariam Ulfah[2,]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang, West Sumatra, Indonesia
[2]Muhammadiyah High School of Health Sciences Cirebon, West Java, Indonesia

*Corresponding author: iryaniachmad6@gmail.com

Received: 9 October 2022; Accepted: 10 June 2023; Published: 25 July 2023

Abstract

BS endophytic Fungi is a Fungi isolated from the flower of the bitter plant (A. Paniculata) which is known to produce various secondary metabolites which are reported to have antibacterial bioactivity. This study aimed to determine the antibacterial activity of extract from endophytic Fungi BS isolated from bitter flower (A. Paniculata) on brown rice growing media against Streptococcus mutans, Staphylococcus aureus, and Pseudomonas aeruginosa bacteria. The method used is endophytic Fungi BS grown on media extracted by maceration method with solvent. The antibacterial activity test of the extract was carried out by disc diffusion method. The results obtained showed that the extract of the endophytic Fungi BS grown on brown rice media gave activity to inhibit the growth of Streptococcus mutans, Staphylococcus aureus, and Pseudomonas aeruginosa bacteria at concentrations of 10%, 30%, and 50%. In conclusion, the extract of BS endophytic Fungi isolated from A. paniculata flowers has potential as an antibacterial.

Keywords: Antibacterial; Brown Rice; Sambiloto Flower; Endophytic Fungi

Introduction

Infectious diseases are diseases that occur due to the growth of pathogenic biological agents in individual host organisms. Infecting pathogens such as bacteria, fungi, viruses, protozoa, and parasites. In Indonesia, infectious diseases are still among the ten most common diseases (Kemenkes RI, 2019). Efforts to treat infectious diseases have been carried out, generally the treatment used is the use of antimicrobials. However, irrational use of antimicrobials both in dose and duration of use can trigger antimicrobial resistance, causing morbidity and mortality. In 2014 it was reported that the death rate due to antimicrobial resistance was 700,000 people per year (Kemenkes RI, 2016).

In order to find alternative treatments for infectious diseases, exploration of the antimicrobial activity of natural materials from both plants and microorganisms is still an option. To overcome this problem, the search for alternative sources that can produce active secondary metabolites is very necessary. One of the potential sources for producing bioactive compounds is endophytic fungi (Sari et al., 2021).

Endophytic fungi are microorganisms that live by colonizing almost all plant tissues, such as roots, flowers, leaves, fruits, and twigs. The relationship between endophytic fungi and their host plants is a

symbiotic mutualism. Endophytic fungi will produce various secondary metabolites with various structures and frameworks in helping their host plants to deal with external attacks. Several groups of secondary metabolites that have been reported from endophytic fungi include alkaloids, steroids, terpenoids, and phenolic derivative compounds with various biological activities (Anshar, et al 2021).

One of the plants that has the potential as a host for endophytic fungi is the *Andrgraphis paniculata* plant, also known as Sambiloto. *A. paniculata* plants have been used traditionally to treat various diseases, such as colds, tonsillitis, intestinal inflammation and fever (Silalahi M, 2020). This ethnopharmacological fact indicates that endophytic fungi associated with *A. paniculata* plants will produce secondary metabolites with various bioactivities, including antibacterial and cytotoxic. Studies related to the chemical content of endophytic fungi that live in the leaves of *A. paniculata* have been previously reported (Yolanda et al 2022). Previous research on endophytic fungi on flower parts of the plant *A. paniculata* with the growing medium used in the form of white rice has also been reported (Suhanah, et al, 2021). However, the antibacterial study of the endophytic fungi associated with the flower parts of the plant *A. paniculata* using a growth medium in the form of brown rice, was the first to be reported in this study. flower of *A. paniculata* plant with growing media in the form of brown rice as a source of antibacterial compounds.

Methods

Tools and materials

The tools used are laminar air flow, petri dish, incubator, erlenmeyer, skewer, autoclave, filter paper, 1 ml syringe, handscon, measuring cup, beaker, measuring cup, stirring rod, separating funnel, petri dish, paper disc, needle ose analytical balance, digital balance, rotary evaporator, tweezers, and caliper.

The materials used consisted of bitter plants (A. paniculata) obtained from the Dadok Tunggul Hitam sub-district, Koto Tangah District, Padang City, ethyl acetate, 70% ethanol, 3.5% NaOCl. Mayer, Wagner, Dragendorf reagents, CHCl3 H2SO4, FeCl3, Brown rice and distilled water Antibacterial test materials PDA (Potato Dextrose Agar) media, distilled water, alcohol, MHA media, disc paper, standard tetracycline (antibiotic), and three test bacteria, namely *Streptococcus mutans, Staphylococcus aureus*, and *Pseudomonas aeruginosa.*

Endophytic Fungi Inoculation

In this study, *A. paniculata* plants as host plants for endophytic fungi were obtained from the Dadok Tunggul Hitam village, Koto Tangah district, Padang City, in April 2021. Then the surface of the fresh *A. paniculata* flowers measuring 2x2 cm was cleaned with water. Furthermore, the flowers are sterilized so that the bacteria found in *A. paniculata* flowers die. The flowers were soaked for 45 seconds in 70% ethanol solution, and NaOCl solution for 30 seconds. Sterile *A. paniculata* flowers were attached to PDA media as a negative control. The sterile *A. paniculata* flower parts were cut into 1x1 cm size to be inoculated on PDA media and incubated at 28oC. Endophytic fungi that grew after 7 days were then sub-cultured to other solid media so that a single isolate of good endophytic fungi was obtained which would be selected for further phytochemical testing (Riga and Hakim, 2021).

Cultivation in Brown Rice Media and Endophytic Fungi Extraction

The BS endophytic Fungi from *A. paniculata* flower that had been obtained was then cultivated in a 250 mL Erlenmeyer containing brown rice which had been cooked in an autoclave at 121oC and 15 lbs pressure for 15 minutes. Then the fermented mushrooms were harvested after reaching the optimum time and continued with the maceration process using ethyl acetate for 3 times where the amount of ethyl acetate used was 50 ml for 1 extraction for 24 hours. The results of the maceration were concentrated to obtain a thick extract of BS endophytic Fungi. The extract of BS

endophytic Fungi from *A. paniculata* flower obtained was tested for its antibacterial activity and secondary metabolite content.

Endophytic Fungi Extract Antibacterial Activity Test

The agar diffusion method was used as an initial test to determine the antimicrobial activity of the compound. Each 24hour old microbe was scratched evenly on the surface of the MHA media. Furthermore, as much as 10 L of endophytic mushroom extract BS (10%, 30% and 50% concentration) because at these concentrations it is effective to test antibacterial activity. The extract was dripped onto blank discs which were placed on the MHA media. The MHA plate was then closed and incubated aerobically at 37°C for 24 hours. The presence of potential antibacterial properties of compounds and extracts was determined from the clear zone around the filter paper. Amoxicillin was used as a positive standard (concentration 0.5%) and a negative control, namely methanol solvent. The test was carried out 3 times.

Secondary Metabolite Content Test

Terpenoid dan Steroid

BS endophytic mushroom extract was put into 3 different test tubes. Then each reaction tube was added with ammonia chloroform and H2SO4 2 N and shaken vigorously, then let stand until two layers were formed. Take the bottom layer and then place it on a drip plate and allow it to evaporate, after drying, anhydrous acetic acid and H2SO4 p.a are added. if the green-blue color is positive for steroids and a positive result for terpenoids it produces a red color.

Alkaloid

The results of the top layer on the steroid and terpenoid test were put into three test tubes. Into each test tube, Dragendorf's reagent, Meyer's reagent and Wagner's reagent were added sequentially. If the brown precipitate, white precipitate and orange precipitate formed is a positive result of alkaloids.

Phenolic Compound

The BS endophytic mushroom extract was placed in 3 holes on the drip plate, then 1% FeCl3 solution was added. If the pink color indicates a positive result of phenolic compounds.

Result and Discussion

A. paniculata is a medicinal plant that contains many bioactivity compounds and one of them is a potential source of antibacterial compounds. *Paniculata* also has various bioactivity compounds. The purpose of this study was to determine the antibacterial potential of endophytic fungi from the part of the bitter flower (*A. paniculata*). *A. Paniculata* flowers that have gone through the sterilization process are inoculated in PDA media that has been added with antibiotics. Usually about a week the endophytic fungi that grow can be sub-cultured to other solid media so as to produce two single isolates of endophytic fungi. Observation of morphology of BS endophytic fungi, which has a morphological form with macroscopic characteristics in the form of white colonies.

Figure 1. BS Eendophytic Fungi

BS Fungi from *A. paniculata* flower was then cultivated on a small-scale measuring 1x1 cm to determine the optimum time for the Fungi to produce secondary metabolites. BS mushroom extracts in the first, second, third and fourth weeks were analyzed to determine their metabolite content. Secondary metabolites are produced by endophytic fungi in the stationary stage, which is the stage where there is a balance between the speed of growth and cell death.

This stage occurs when the nutrients contained in the media begin to run out. so that it will cause enzymes that play a role in the production of secondary metabolites to accumulate and secondary metabolites to be produced in greater quantities (Aulia Suhanah R, 2021).

BS mushrooms were further cultivated on a large scale into 25 Erlenmeyer 25 mL size containing rice media. Large-scale cultivation was carried out to obtain a larger amount of extract mass. After being cultivated during the stationary stage (3 weeks), BS mushrooms were harvested and extracted using ethyl acetate as solvent. The extraction results were concentrated to obtain a concentrated extract of BS endophytic Fungi.

The concentrated extract of the BS endophytic Fungi was tested for its antibacterial activity using the disc diffusion method. The antibacterial activity was tested against three test bacteria, namely *Streptococcus mutans, Staphylococcus aureus*, and *Pseudomonas aeroginosa*. The concentration of the tested extracts consisted of three variations, namely 10%, 30% and 50%. The positive control used in this antibacterial test was amoxicillin. The antibacterial activity test was carried out in triples with the aim of obtaining confidence in the results of the analysis. and the test results are expressed as zones of inhibition which are shown in Table 1.

Table 1 shows that the BS mushroom extract isolated from *A. paniculata* flowers had the ability to inhibit the growth of all the tested bacteria. The higher the concentration of the extract was positively correlated with its ability as an antibacterial agent. This is because the content of active compounds is getting bigger. The increasing content of active compounds will make the ability of the extract to inhibit the test bacteria also increase.

Table 1. Inhibition Zone Data of BS Endophytic Fungi Extract Against Test Bacteria

Test Bacteria	Inhibition Zone Diameter			
	10%	30%	50%	Kontrol +
Pseudomonas aeroginosa	6,86±0,58	6,86±0,58	8,90±0,47	16,66±0,25
Streptococcus mutans	6,48±0,46	6,99±0,64	12,55±0,10	17,77±0,41
Staphylococcus aureus	8,69±0,23	8,87±0,11	12,55±0,10	16,63±0,31

The positive results of the antibacterial test on the extract indicated the presence of secondary metabolites that have potential as antibacterial compounds. Furthermore, the extract was tested for the content of secondary metabolites which include alkaloids and terpenoids. The test results for the chemical content of organic extracts are shown in Table 2.

The classification of the response to bacterial growth inhibition according to Davis and Stout (1971) is that if the diameter of the inhibition zone is >20 mm, the inhibition response is very strong, if the diameter of the inhibition zone is 10-20 mm, it is strong, 5-10 mm is moderate and if diameter <5 mm is classified as having a weak bacterial growth inhibitory response. While the results of this study showed that the extract of the endophytic Fungi BS from the sambiloto plant *A. paniculata* which was grown on brown rice growing media, contained strong antibacterial activity because according to the diameter of the bacterial inhibition zone in this study, the extract of the endophytic Fungi BS grown on brown rice media had diameter >6-12mm

The antibacterial activity test in this study was carried out three times (triplo) on the bacteria *Streptococcus mutans* and *Pseudomonas aeruginosa* why use this bacterium because *Streptococcus mutans* is one of the microorganisms found on the surface of the oral cavity. on the tooth surface. *Streptococcus mutans* can stick to and is able to hydrolyze food debris that is between the teeth. This results in the occurrence of bacteria on tooth enamel and

plaque is formed as the initial formation of dental caries. In addition, the presence of plaque can also cause an unpleasant odor in the mouth (Mayasari & Sapitri, 2019)

P. aeruginosa is one of the most common gram-negative bacteria found in the normal flora of human skin and intestines. Pratami et al. (2013) reported that P. aeruginosa bacteria were found mostly due to contamination of polluted water used for washing hands. Purwani et al. (2012) stated that the bacterium P. aeruginosa is one of the food-destroying microorganisms that can be found in fish and meat. *P. aeruginosa* bacteria are reported as one of the bacteria that are resistant to antibiotics (Deni and Pangalila, 2019). *Staphylococcus aureus* is a gram-positive, coccus-shaped bacterium and is a pathogenic bacterium for humans.

Staphylococcus aureus is a bacterium that can infect tissues and organs of the body so that it can cause abscesses, various pyogenic infections (such as endocarditis, septic arthritis, and osteomyelitis), food poisoning, scalded skin syndrome, and toxic shock syndrome. This bacterium is also one of the most common causes of pneumonia, septicemia, and surgical wound infections Therefore, this bacterium is an important cause of skin infections, such as folliculitis, cellulitis, and impetigo (Kinanty, 2015).

On disc paper dripped with methanol solvent (negative control) there was absolutely no inhibition zone for bacterial growth, while on disc paper dripped with amoxicillin solution diluted (amoxicillin 0.5%) with methanol solvent had a very large diameter inhibition zone, namely >16-17 mm. In the BS endophytic fungal extract solution, the average diameter of the bacterial inhibition zone was that on paper discs containing 50% BS endophytic Fungi extract, the inhibitory power was greater than that of disc paper containing 30% and 10% BS endophytic Fungi extracts. This is because the 50% extract concentration of secondary metabolites is greater than the 30% and 10% extracts.

The results of the phytochemical test of BS endophytic mushroom extract on brown rice growing media were that it contained alkaloids and terpenoids (as shown in table 2). The mechanism of action of secondary metabolites in the alkaloid group in inhibiting bacterial growth is by interfering with the constituent components of peptidoglycan in bacterial cells. Meanwhile, non-phenolic compounds such as terpenoids have antibacterial activity by interfering with the process of membrane or cell wall formation. This mechanism occurs in the outer membrane of the bacterial cell wall, the terpenoid compounds contained in the BS endophytic fungal extract will react with the porin (transmembrane protein) to form strong polymer bonds that can damage the porin so that it can inhibit bacterial growth. This causes the cell wall or membrane will not be formed completely.

The difference in the sensitivity of bacteria to antibacterials is influenced by: bacterial cell wall structure. Gram-positive bacteria tend to be more sensitive to antibacterial, because the cell wall structure of gram-positive bacteria is simpler compared to the structure of the cell wall of gram-negative bacteria (as in Figure 2), making it easier for antibacterial compounds to enter bacterial cells gram positive. According to the results of research conducted by Ningtyas et al. (2012) While in this study *Staphylococcus aureus* and *Streptococcus mutans* had a gram-positive cell wall structure and *Pseudomonas aeruginosa* had a gram-negative cell wall structure. It can be seen in table 1. The average diameter of the growth inhibition zone of *Staphylococcus aureus* and *Streptococcus mutans* bacteria is greater than that of in negative cell wall bacteria, namely *Pseudomonas aeruginosa*.

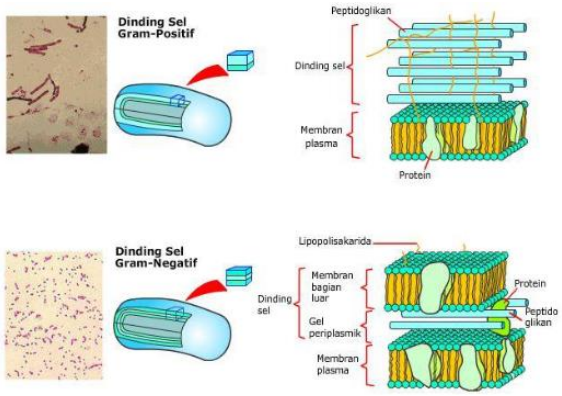

Figure 2. The Mechanism of Action of Bacterial Cell wall Destruction (Ningtyas *et al.,*2012)

Research related to secondary metabolites that play a role in antibacterial activity in BS mushroom extract needs to be continued so that it can complete information related to antibacterial compounds from BS mushrooms.

Table 2. Phytochemical Test Results of BS Endophytic Fungi

Secondary Metabolites	Reactor	The Results	Description
Terpenoid	$CHCl_3$ dan $H2SO_4$	+	Red Color is Formed
Alkaloid	Mayer	+	A white precipitate is formed
	Wagner	+	A brown precipitate is formed
	Dragendorf	-	No orange precipitateis formed
Steroid	$CHCl_3$ dan $H2SO_4$	-	No green-blue precipitate is formed
Phenolic	$FeCl_3$	-	No blue-black color is formed

Conclusion

Based on the results of this study, it can be concluded that the antibacterial activity test against three test bacteria, *Streptococcus mutans, Staphylococcus aureus, and Pseudomonas aeroginosa*, namely BS endophytic mushroom extract isolated from *A. paniculata* flowers can inhibit bacterial growth. The greater the concentration of the extract, the greater the inhibition zone obtained.

References

Ansar, Varel Alkhairi, Sri Benti Ethics, Suryelita, Ulfah Mariam, Riga Riga (2021). Study of the antibacterial activity of endophytic fungi that colonize the twigs of *Andrographis paniculata.* 21(02), 137–144.

Davis, W. W. and T. R. Stout. 1971.Disc plate methods of microbiological antibiotic assay. Microbiology 22: 659-665.

Deni J, Pangalila FJV. 2019. The relationship between successful therapy of Pseudomonas aeruginosa and Acinetobacter baumannii-resistant nosocomial pneumonia with a dose of Carbapenem in the ICU of Royal Taruma Hospital for the period 2012-2017. Tarumanagara Medical Journal. 2(1): 65-76.

Ministry of Health (Kemenkes) RI. (2016). Data and Information: Indonesia Health Profile 2016.

https://pusdatin.kemkes.go.id/article/view/17092000001/profil-kesehatan-indonesia-2016.html. Accessed: March 10, 2020.

Ministry of Health (Kemenkes) RI. (2019). Main Results of Riskesdas 2018 by the Ministry of Health of the Republic of Indonesia.

ttps://kesmas.kemkes.go.id. Accessed: 21 Mei 2020

Kinanty Anisa. 2015. Antibacterial Activity Test of Shredded Banana (Musa paradisiaca) Ethyl Acetate Extract Against Staphilococcus aureus. Thesis. Not published. Medical School. Tanjungpura University: Langkat.

Mayasari Ulfayani & Sapitri Alfi. 2019. Antibacterial Activity Test of Fragrant Lemongrass Leaves (Cymbopogen nardus) Against *Streptococcus mutans* Bacterial Growth. Chlorophyll Journal, vol 3no2.,2019:15-19 ISSN 2598-6015

Ningtyas. A. Intan Lestari, 2012 Differences in Concentration and Antibacterial Activity Test of Ethanolic Extract of Batang Kluthuk (Musa balbisiana colla) Against Staphilococcus aureus and Pseudomonas aeruginosa. Thesis. Not published. Faculty of Math and Science. Eleven Maret University, Surakarta.

Purwani E, Retnaningtyas E, Widowati D. 2012. Development of a natural preservative model from extracts of galangal (Languas galanga), turmeric (Curcuma domestica) and ginger (Zingiber officinale) as a substitute for formalin in fresh meat. Proceedings of Biology Education Centre. 9(1): 629-634

Riga, and Euis Holisotan Hakim. 2021. "Cytotoxic and Antibacterial Activity of Ethyl Acetate Extract of Endophytic Fungi Colletotrichum Gloeosporioides." Journal of Pharmacy Udayana 10(2):193.

Sari, N. P. D. P., Cahyo, B. D., Sugijanto, N. E. N., & Suciati, S. (2021). Antibacterial activity of Endophytic Fungi Penicillium oxalicum Isolated from Sponge Homaxinella tanitai. Indonesian Journal of Pharmacy and Pharmaceutical Sciences, 8(1), 10. https://doi.org/10.20473/jfiki.v8i12021.10-15

Suhanah R, A., Suryelita, Sri Benti Ethics, Ulfah Meriam, Riga. (2021). Endophytic Fungi Isolated from *Andrographis Paniculat*a (Sambiloto) Flower As A Source Of Antibacterial Compounds. Indonesian Journal of Pharmacy Personnel, 4(1), 139–148.
https://doi.org/10.36387/jifi.v4i1.664

Silalahi, M. (2020). Sambiroto *Andrographis paniculata*) and its bioactivity. BEST Journal (Biology Education, Science and Technology), 3(1), 76–84.
https://doi.org/10.30743/best.v3i1.2448

Wikler, M. A., Cockerill, F. R. & Bush, K. D. (2009). Performance Standard for Antimicrobial Disk Susceptibility Test; Approved Standard (10th ed.). Pennsylvania: Clinical and Laboratory Standards Institute.S.

Yolanda Melisa, S., Ethics, Riga. 2022, Study of Phytochemical and Antibacterial Properties of Endophytic Fungi RS-1 on *Andrographis paniculata* (Sambiloto) Twigs with Brown Rice Growth Media, Journal of Education, Mathematics and SCIENCE. 7(1), 91–98.

Zhang, C. Zhu, J. K. O. Sin, and P. K. T. Mok, "A novel ultrathin elevated channel low-temperature poly-Si TFT," *IEEE Electron Device Lett.*, vol. 20, pp. 569–Nov. 1999.