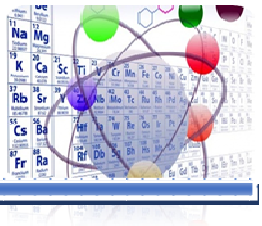
Indonesian Journal of Chemical Science and Technology (IJCST)

State University of Medan, https://jurnal.unimed.ac.id/2012/index.php/aromatika

IJCST-UNIMED 2023, Vol. 06, No. 1 Page; 40 – 44

Received : Dec 21th, 2022 Accepted : Jan 5th, 2023 Web Published : Jan 31st, 2023



Effectiveness Test of the Antibacterial Activity of Endophytic Fungi RS-2 from Sambiloto (*Andrographis paniculata*) on Red Rice Media

Luqiyatur Rohmah[1*], Suryelita Suryelita[1], Riga Riga[1] dan Mariam Ulfah[2]

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

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

** Corresponding author : luqiyaturr@gmail.com*

ABSTRACT

A. paniculata *(Sambiloto) is a plant capable of producing various secondary metabolites which use in the field of pharmacology, one of which is as a source of antibacterial compounds. This potential can be explored from endophytic fungi associated with* A. paniculata*. The purpose was to determine the effectiveness of the antibacterial activity of the endophytic fungi RS-2 from branches of* A. paniculata *using red rice as the media. The stages of the research included isolation of the fungi, cultivation, extraction, antibacterial test, and test of the phytochemical of the endophytic fungi on branches of* A. paniculata*. The effectiveness test of antibacterial activity against the extract of the RS-2 endophytic fungi showed that the endophytic fungi extract had the potential to inhibit the growth of the three tested bacteria at concentrations of 10%, 30%, and 50%. The ethyl acetate extract of the endophytic fungi RS-2 from* A. paniculata *has the potential as a source of antibacterial compounds using red rice media.*

Keywords: *A. paniculata,* antibacterial, endophytic fungi, RS-2

1. INTRODUCTION

Antibiotics are antibacterial drugs that have been widely used by the world community since 70 years ago. Antibiotics can treat various infectious diseases caused by bacteria and in their development, resistance to various antibiotics occurs due to inappropriate use of antibiotics for diseases that are not in accordance with a doctor's prescription.[1] Based on this, the search for alternative sources that can produce secondary metabolites that have the potential as anti-bacterial is urgently needed, one of these alternatives is the culture of endophytic fungi.

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

Endophytic fungi are microorganisms that live and form colonies in plant tissues without harming their hosts. This fungus is capable of producing the same or even different bioactive compounds from its host plant.[3] Host plants can provide endophytic fungi with nutrients, media, and shelter for reproduction, while endophytic fungi will produce bioactive compounds that can protect and defend host plants from various factors. So a mutualism symbiosis is formed between endophytic fungi and their host plants.[4]

Endophytic fungi associated with medicinal plants have great potential in producing bioactive compounds such as terpenoids, steroids, alkaloids, and phenolic compounds with various biological activities. One of these medicinal plants is Sambiloto. Sambiloto (A. paniculata) is a herbal plant that is widely used in the traditional medicine industry in Indonesia.[5] Twigs, stems, branches, leaves, and bitter roots are useful for curing typhoid, antibacterial, anti-diarrhea, tuberculosis (tuberculosis), whooping cough, gonorrhea, fever, and liver disorders.[6]

Research on phytochemical studies and antibacterial effectiveness of the endophytic fungi RS-2 branch of A. paniculata using white rice media has been reported previously.[7] Studies regarding the antibacterial effectiveness of the RS-2 endophytic fungi A. paniculata using red rice media have not previously been carried out. Based on this, it is necessary to conduct this research to explore the potency of the endophytic fungi RS-2 in the internal tissues of A. edpaniculata branches as an antibacterial compound in red rice media.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The materials used in this study were the bitter plant (A. paniculata), Potato Dextrose Agar (PDA), brown rice, ethyl acetate, 70% ethanol, 3.5% NaOCl, distilled water, methanol, Mueller Hinton Agar (MHA), discs paper, amoxicillin, and three test bacteria namely Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans. The tools used are a petri dish, Erlenmeyer, measuring cup, stirring rod, tweezers, paper filters, autoclave, incubator, Laminar Air Flow, loop needle, analytical balance, digital scale, caliper, and rotary evaporator.

2.2. Research Procedure

2.2.1 Endophytic Fungus Inoculation

The A. paniculata used in this study was obtained from Banda Gadang Tabing, Nanggalo District, Padang City. A. paniculata twig measuring 2x2cm was washed with clean running water. Next, the surface of the twigs was sterilized by immersing the twigs for 45 seconds in a 70% C2H5OH solution and for 30 seconds in a 3.5% NaOCl solution. Sterilization was carried out to kill epiphytic microbes on the surface of A. paniculata twigs. After the sterilization process, twigs were attached as negative controls to PDA solid media. A. paniculata twigs were cut into 1x1cm sizes and inoculated in PDA media and then placed in an incubator at 28℃. Endophytic fungi that grew after seven days were sub-cultured on new media so that a single isolated endophytic fungus was obtained.

2.2.2 Cultivation and Manufacturing of Endophytic Fungus Extracts

Endophytic fungi from PDA media were cultivated on red rice media in 250 mL Erlenmeyer and incubated during the cultivation optimization time at 28℃. The endophytic fungi were then harvested and

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

extracted for 3x24 hours with ethyl acetate solvent. The results of the concentrated extract were then tested for the effectiveness of antibacterial activity and phytochemical tests.

2.2.3 Secondary Metabolite Content Test

Terpenoid/steroid

RS-2 endophytic fungi extract was put in a test tube. Ammonia-chloroform and 2N H2SO4 were added to a test tube containing the extract, then shaken and allowed to stand. After two layers were formed, the bottom layer was taken and transferred to the drip plate, left until the solvent evaporated, and then dripped with an acetic acid anhydride and H_2SO_4 p.a. Formation of red color indicates positive terpenoids and blue-green indicates positive steroids.

Alkaloids

In three different test tubes, the top layer of the terpenoid test was added. Each test tube was added to the first tube of Wagner's reagent, the second tube of Mayer's reagent, and the third tube of dragendorf's reagent. The orange precipitate, white precipitate, and brown precipitate respectively showed positive alkaloids.

Phenolic

The extract of RS-2 endophytic fungi was put into the drip plate, then 1% FeCl3 was added. Positive phenolic compounds are indicated by a pink color change.

2.2.4 Antibacterial Activity Test

Antibacterial activity test was carried out using P. aeruginosa, S. aureus, and S. mutans bacteria. A total of 10 µL concentrated extract with various concentrations, namely 10%, 30%, and 50% (solvent: methanol). Dropped on amoxicillin disc paper as a positive control and methanol as a negative control over each of the test bacteria. The antibacterial activity of each extract was measured after incubation for 1x24 hours and expressed in the inhibition zone. The test process was carried out under aseptic conditions and the antibacterial activity was carried out in triples.

3. RESULTS AND DISCUSSION

A. paniculata has many uses in the health sector which can cure various diseases. This is due to the presence of bioactive compounds in A. paniculata so that the associated endophytic fungi in plant tissues can also produce similar bioactive compounds. Sterile A. paniculata twigs were inoculated on PDA solid media which had been added with antibiotics. The addition of antibiotics was carried out to kill bacteria present in the plant branch tissue. Single isolates of endophytic fungi produced after sub-culture were coded RS-2 and then processed in the next stage

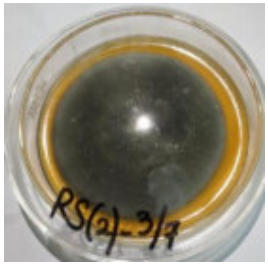


Figure 1. Endophytic fungi morphology RS-2

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

The shape of the RS-2 endophytic fungi, as seen in Figure 1, has macroscopic characteristics, including green-black colonies, round shapes, and forming concentric colonies. The macroscopic morphology of the RS-2 branch of A. paniculata has never been reported. The endophytic fungi RS-2 was cultivated on a large scale in red rice media in 10 Erlenmeyer 250 mL. Then harvested after two weeks which is the optimum time and extracted with ethyl acetate. The extract obtained was then concentrated. The concentrated extract was followed by testing for secondary metabolites, including terpenoids, steroids, alkaloids, and compounds, which can be seen in table 1..

Table 1. Secondary metabolite content test results

No.	Chemical Compound	Reactor	Test results
1.	Alkaloids	Dragendorf	+
		Wagner	+
		Mayer	-
2.	Terpenoids	$CHCl_3$ and H_2SO_4	+
3.	Steroids	$CHCl_3$ and H_2SO_4	-
4.	Phenolic	$FeCl_3$	+

Then tested the antibacterial activity. The ethyl acetate extract was tested for antibacterial activity against P. aeruginosa, S. aureus, and S. mutans bacteria by varying the concentration of the RS-2 endophytic fungi extract with concentrations of 10%, 30%, and 50%. The results of the antibacterial test were expressed in the inhibition zone which was carried out in triple

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

Concentration	Test bacteria		
	P. aeruginosa	***S. aureus***	***S. mutans***
10%	6,06±0,39	9,01±0,69	7,95±0,61
30%	7,82±0,60	11,09±0,65	9,56±0,54
50%	11,86±0,29	14,24±0,39	15,19±0,66
Positive Control (+)	15,71±0,51	18,53±0,51	19,21±0,27

Table 2 shows that the ethyl acetate extract of the endophytic fungi RS-2 branch of A. paniculata has the potential to inhibit the growth of the three tested bacteria. The high concentration of the extract causes a good mutual relationship with its potential as an antibacterial agent. The increased potency of the extract in inhibiting the growth of the tested bacteria is due to the increasing content of the active compound [9]. The mechanism of action of secondary metabolites varies in inhibiting bacterial growth. In the alkaloid group, it inhibits peptidoglycan in cells by interfering with its constituent components. Terpenoid/steroid compounds inhibit by damaging the formation of the cell wall or membrane, thus causing the cell wall or membrane not to form completely.[10] Phenolic compounds can prevent the formation of bacterial cells by inhibiting the work of reverse transcription enzymes and DNA topoisomerase.[11]

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

4. CONCLUSION

Antibacterial test of the ethyl acetate extract of RS-2 endophytic fungi with red rice media against the test bacteria namely P. aeruginosa, S. aureus, and S. mutans has the potential to inhibit the growth of the test bacteria.

ACKNOWLEDGEMENT

On this occasion, the authors would like to thank Mrs. Dra. Suryelita, M.Si, Dr. Riga, S.Pd, M.Si, and Mrs. Mariam Ulfah who have helped in this research.

REFERENCES

1. Desrini S. (**2015**). Resistensi Antibiotik, Akankah Dapat Dikendalikan ? *J. Kedokt. dan Kesehat. Indones.*, vol. 6, no. 4, pp. i–iii.
2. Yarza H. L., Yanwirasti Y., and Irawati L. (**2015**). Hubungan Tingkat Pengetahuan dan Sikap dengan Penggunaan Antibiotik Tanpa Resep Dokter. *J. Kesehat. Andalas*, vol. 4, no. 1, pp. 151–156.
3. Radji M.. (**2005**). Peranan Bioteknologi Dan Mikroba Endofit dalam Pengembangan Obat Herbal. *Maj. Ilmu Kefarmasian*, vol. 2, no. 3, pp. 113–126.
4. Riga. (**2017**). Isolasi dan Karakterisasi Metabolit Sekunder dari Colletotrichum gloeosporioides, Jamur Endofitik Tumbuhan Artocarpus heterophyllus, Institut Teknologi Bandung
5. Pagare S., Bhatia M., Tripathi N., Pagare S., and Bansal Y. K. (**2015**). Secondary metabolites of plants and their role: Overview. *Curr. Trends Biotechnol. Pharm.*, vol. 9, no. 3, pp. 293–304, 2015.
6. Malik N. (**2014**). Pertumbuhan Tinggi Tanaman Sambiloto (Andrographis paniculata Ness) Hasil Pemberian Pupuk dan Intensitas Cahaya Matahari yang Berbeda The Growth of Plant Height of Bitter Plant (Andrographis paniculata Ness) with the Addition of Fertilizer and Differe, *J. Agroteknos*, vol. 4, no. 3, pp. 189–193.
7. Anshar V., Khairi A., Etika S. B., Suhanah R. A., and Riga R. (**2022**). Aktivitas Antibakteri Jamur Endofitik RS-2 yag Diisolasi dari Tumbuhan Sambiloto (Andrographis paniculata) Antibacterial Activity of Fungus RS-2 Isolated from Sambiloto (Andrographis paniculata). vol. 10, no. 1, pp. 1–5.
8. Riga R., Happyana N., Quentmeier A., Zammarelli C., Kayser O., and Hakim E. H. (**2021**). Secondary metabolites from Diaporthe lithocarpus isolated from Artocarpus heterophyllus. *Nat. Prod. Res.*, vol. 35, no. 14, pp. 2324–2328.
9. Riga R.and Hakim E. H. (**2021**). Aktivitas Sitotoksik dan Antibakteri Ekstrak Etil Asetat Jamur Endofitik Colletotrichum gloeosporioides yang Diisolasi dari Daun Artocarpus heterophyllus. *J. Farm. Udayana*, vol. 10, no. 2, p. 193.
10. Kurniawan B. and Aryana W. F. (2015). Binahong (Cassia Alata L) As Inhibitor Of Escherichiacoli Growth," vol. 4, pp. 100–104
11. Varel Anshar R.R. Al Kh.airi , Sri Benti Etika , Suryelita Suryelita, Mariam Ulfah. (**2021**). Study of The Antibacterial Activity of Endophytic Fungus That Colonize With The Twig of Andrographis paniculata, *Eksakta*, vol. 22 NO 02.

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