Antibacterial Activity of The Secondary Metabolites Produced by Endophytic Fungi Isolated from Acanthus ilicifolius Plant

by Salni Salni

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Antibacterial Activity of The Secondary Metabolites Produced by Endophytic Fungi Isolated from *Acanthus ilicifolius* Plant

Gusti Ayu Widayanti[1], Hary Widjajanti[2], Salni[2]*,

[1]*Department of Biosciences. Biology Program. Faculty of Science. Sriwijaya University. Jalan Padang Selasa 524. Palembang. South Sumatra 30139. Indonesia.*
[2]*Department of Biology. Faculty of Mathematics & Natural Sciences. Sriwijaya University. Jalan Raya Palembang-Prabumulih km 32. Indralaya. Indonesia.*
***Corresponding author e-mail**: awegusti@gmail.com

Abstract
Endophytic fungi can produce secondary metabolites that are same as the host. Twelve endophytic fungi were isolated from the *Acanthus ilicifolius* plant. Two of them have antibacterial activity, DJS1 and DJS2. Fungi can produce secondary metabolites that have the potential as antibacterial compounds. This study aims to isolate antibacterial compounds using column chromatography methods and test the antibacterial activity using diffusion methods to use paper discs. Isolation of secondary metabolites produces 27 eluates. From TLC, eluate $DJS1_1$ and eluate $DJS2_2$ contains phenol compound, eluate $DJS2_4$ contains terpenoids compound, and eluate $DJS2_5$ contain alkaloids copound, each component of the active compound are tested for sensitivity to bacterias *Escherichia coli, Staphylococcus aureus, Shigella dysenteriae*, and *Salmonella typhi*. MIC value of *Escherichia coli* on $DJS1_1$ eluate is 500 ppm, $DJS2_2$ eluate is 250 ppm, $DJS2_4$ eluate is 250 ppm, and $DJS2_5$ eluate is 250 ppm. MIC value of *Staphylococcus aureus* on $DJS1_1$ eluate is 500 ppm, $DJS2_2$ is 1000 ppm eluate, $DJS2_4$ eluate is 125 ppm, and $DJS2_5$ eluate is 500 ppm. MIC value of *Shigella dysenteriae* on $DJS1_1$ eluate is 500 ppm, $DJS2_2$ eluate is 1000 ppm, $DJS2_4$ eluate is 250 ppm, and $DJS2_5$ eluate is 500 ppm. MIC value of *Salmonella typhi* on $DJS1_1$ eluate is 125 ppm, $DJS2_2$ eluate is 125 ppm, $DJS2_4$ eluate is 125 ppm, and $DJS2_5$ eluate is 125 ppm.

Keywords
Acanthus ilicifolius, MIC, Antibacterial compound

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1. INTRODUCTION

Antibiotics are needed to overcome diseases caused by bacterial infections (Septiani et al., 2017b). Consumption of antibiotics in high intensity can cause various kinds of problems, especially bacterial resistance to antibiotics (Utami, 2012). Based on these problems, other sources of antibacterial are sought to overcome these problems by utilizing medicinal plants. Efforts to improve the efficiency of utilization of medicinal plants are carried out using microbiological biotechnology approaches, namely through the use of endophytic fungi from plants.

Biosynthesis of secondary metabolites from endophytic fungi that have the potential as an antibacterial compound has been widely carried out before, extracts of endophytic fungi from *Rizophora apiculata* plant has very good antibacterial activity with the formation of inhibition zones of 12.07 mm in *Escherichia coli* and 14.62 mm in *Staphylococcus aureus* (Mukhlis et al., 2018). Other similar studies such as having *Sonneratia alba* plant has an inhibition zones of 14.7 mm in *Staphylococcus aureus* and 11.7 mm in *Escherichia coli* (Nawea et al., 2017) and Mangifera indica L plant has an inhibition zones of 18.39 nm in *Staphylococcus aureus*, 15.68 mm in *Salmonella typhi*, 26.23 *Streptococcus mutans*, and 22.38 in *Escherichia coli* (Rosalina et al., 2018). This proves that endophytic fungi can produce compounds that have the potential to be antibacterial.

Endophytic fungi are isolated from the stems, leaves, and roots of the *Acanthus ilicifolius* plant. This study aims to reveal the antibacterial activity of fungi and find secondary metabolite compounds that act as antibacterial compounds using contact bioautography and thin-layer chromatography methods. The results are expected to provide information about alternative antibacterial compounds produced from endophytic fungi.

2. EXPERIMENTAL SECTION

2.1 Materials

Leaves, stems, and root *Acanthus ilicifolius* plant. The bacterial (*Staphylococcus aureus, Escherichia coli, Shigella dysentriae, Salmonella typhi*) used for the research of antibacterial activity were acquired from Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University. Sodium chloride (NaCl). Potato Dextrose Agar (PDA), medium Potato Dextrose Borth (PDB), medium Nutrient Agar (NA), Nutrient Borth (NB), medium Mueller Hinton Agar (MHA), silica gel, TLC silica gel 60 F254 , paper disc, n-hexane, ethyl acetate, methanol, dimethyl sulfoxide (DMSO), sulfuric acid (H_2SO_4), all from Merck.

2.2 Methods

2.2.1 Isolation and culturing of the endophytic fungi

Acanthus ilicifolius plant collected from Sungsang, Sumatera Selatan. The fresh leaves, stems, and root parts were used for the isolation of endophytic fungi are stored in the icebox at 4 °C which was previously packaged in sealed plastic bags. Healthy mature leaves, stems, and root of Acanthus ilicifolius plant were washed thoroughly under running tap water, then the samples were sterilized by dipping them in ethanol 75%, followed by immersing in 3% NaOCl, dipping them in 75% ethanol, then rinsed in sterile distilled water, and finally dried on sterile filter paper on a petri dish. A piece of each sample was removed with a sterile scalpel then cut into small pieces about 0.5x2 cm, each piece was put on a petri dish containing Potato Dextrose Agar (PDA) medium and incubated at room temperature (28 °C) to promote fungal growth and sporulation (Katoch et al., 2014; Fitriarni and Kasiamdari, 2018). After 7 days, individual hyphal tips of actively growing fungi were picked up for subculturing by inoculating it onto a new PDA medium plate individually and incubated at room temperature for one week. The purified fungal isolates were labeled for further used.

2.2.2 Cultivation and extraction of the secondary metabolites of the endophytic fungi

A total of 15 pieces of agar containing mycelium are cut using a cork borer and inoculated into 500 ml Potato Dextrose Broth (PDB), conditions must be sterile. The culture was incubated at room temperature (28 °C) for 30 days. Sterile PDB is used as a control. The medium PDB is separated from the fungi using filter paper. Weight of fungi biomass and dry biomass were measured (Vinale et al., 2006).

The media is mixed with ethyl acetate to extract secondary metabolites, the use of ethyl acetate aims to attract polar and non-polar compounds. Shake the mixture of medium and ethyl acetate became layers. The first layer (top) is the medium layer and the second layer (bottom) is the layer of ethyl acetate solvent containing secondary metabolites. The ethyl acetate layer containing secondary metabolites was extracted using a rotary evaporator at 80 °C to obtain a thick extract from endophytic fungi (Sharma et al., 2016).

2.2.3 The isolation of antibacterial compounds

The secondary metabolites in extracts were isolated by column chromatography. The silica gel powder $60F_{254}$ poured into the column and stirred to avoid the air cavity in the middle of the column. The silica gel powder in the column reached 14 cm and added the extract. The n-hexane: ethyl acetate and ethyl acetate : methanol solvent with the ratio of 9:1 ; 8:2 ; 7:3 ; 6:4 ; 5:5 ; 4:5 ; 3:7 ; 2:8 ; 1:9 as much as 50 mL were poured into the column gradually, with the column tap opened. The solvent from the column tab was stored in vial 20 mL and evaporated using a hair dryer until eluate was obtained. Each eluate will be tasted for antibacterial activity (Salni et al., 2011).

2.2.4 Detection of antibacterial activity compounds

The antibacterial activity in the eluate compound was tested using the diffusion method to use paper discs. Each eluate was diluted with methanol and paper discs were poured into it. The paper disk is left saturated. A 0.1 mL bacterial suspension was poured into a sterile petri dish. Sterile Mueller Hinton Agar (MHA) is poured into a petri dish containing a bacterial test suspension and allowing it to freeze. The paper disk placed at the MHA was then incubated at 37 ºC for 24 hours and observed, the clear zone showed the eluate had antibacterial compounds. The clear zone is measured using a caliper (Saptiani et al., 2013).

2.2.5 Thin Layer Chromatography (TLC)

The four most active eluates are diluted using methanol and each eluate is bottled on a plate, and let the methanol evaporate. The plate was eluted by eluent (a mixture of organic solvent). The plate was observed on UV light (254nm). Then, the plate was sprayed with 10% H_2SO_4 and heated on a hot plate. The color spot appeared was observed and the Rf value was determined using this formula (Pyka, 2014).

$$Rf = \frac{Distancetravelledbycomponent}{Distancetravelledbysolvent}$$

2.2.6 Detection of antimicrobial activity by bioautography

Detection of the antimicrobial activity of the eluate compound by bioautography-contact method. In bioautography-contact, diffuse antimicrobial agents from a developed plate or paper to an inoculated agar plate. The plate is placed face down onto the inoculated so that the layer for a specific period to enable diffusion. The plate is removed and the order layer is incubated at 37 °C for 18-24 hours. Then the inhibition zone is formed. The zones of inhibition on the agar surface, corresponding to the spots in the chromatographic plates are indicative of antimicrobial substances (Dewanjee et al., 2015).

2.2.7 Determination of Minimum Inhibitory Concentration (MIC)

Eluate compounds which have the ability to inhibit the growth of *Escherichia coli, Staphylococcus aureus, Salmonella typhi*, and *Shigella dysenteriae* were chosen to get the determination of Minimum Inhibitory Concentration (MIC). MIC determination was carried out by the diffusion method using paper discs with a diameter of 6 mm. Isolate compounds were made with concentrations of 1000 ; 500 ; 250 ; 125; 62.5 ; 31.25 ppm used DMSO as a solvent, the control was dropped on paper discs and placed on the Mueller Hinton Agar (MHA) medium (Salni et al., 2011). The 0.1 mL bacterial was poured into the sterile petri dish. The 10 mL sterile MHA was poured into a petri dish which had contained the bacterial test suspension and let it solidify. The paper disc placed on MHA. The petri dish was incubated at 37 °C for 24 hours and observed, clear zone showed the eluate has antibacterial compound. The clear zone was measured using calipers (Saptiani et al., 2013).

2.2.8 Identification of the endophytic fungi

The endophytic fungi produce active compounds as antibacterial were identified to determine the type of endophytic fungi. The identification of endophytic fungi was identified in the Division of Genetics Lab, PT. Genetics Science Indonesia, West Jakarta. Identification using molecular techniques involving polymerase chain reaction (PCR) and DNA sequencing was carried out. Identification using ± 700bp DNA fragments and ITS primers.

3. RESULTS AND DISCUSSION

The results of endophytic fungi derived from the roots, stems, and leaves of *Acanthus ilicifolius* taken from Sungsang, South Sumatra, produce twelve types of endophytic fungi namely, one from the root (AJS1), five from the leaf (BJS1), (BJS2), (BJS3), (BJS4), and (BJS5), and six from the stem (DJS1), (DJS2), (DJS3), (DJS4), (DJS5), (DJS6). This shows that every part of the vascular plant must have endophytic fungi. This is consistent with previous reports that all plant samples were found to accommodate various endophytic fungi with different levels of colonization (Wiyakrutta et al., 2004; Strobel, 2003). All endophytic fungi from *Achantus ilicifolius* can be seen in Figure 1.

Cultivation aims so that endophytic fungi can produce secondary metabolites. Secondary metabolites contain many various compounds which have antibacterial activity (Sharma et al., 2016). After 30 days, the fungi mycelium in the medium increases, it means the fungi grows. The fungi grows because it is successfully adapted to the medium. Adaptation makes the fungi absorb nutrients such as carbon. Adaptation can harmonize fungal physiology under medium environmental conditions (Muthu and Shanmugasundaram, 2015). The changes in the PDB are caused the fungi cultivated produce secondary metabolites as a result of their metabolic processes, the secondary metabolites are produced

Figure 1. *Acanthus ilicifolius* endophytic fungi isolates AJS1 (A), BJS1 (B), BJS2 (C), BJS3 (D), BJS4 (E), BJS5 (F), DJS1 (G), DJS2 (H), DJS3 (I), DJS4 (J), DJS5 (K), dan DJS6 (L)

by fungi at the end of the stationary phase. Synthesis of secondary metabolites will begin when there is limited nutrition in growth media (VanderMolen et al., 2013). Changes in PDB can be seen in Figure 2.

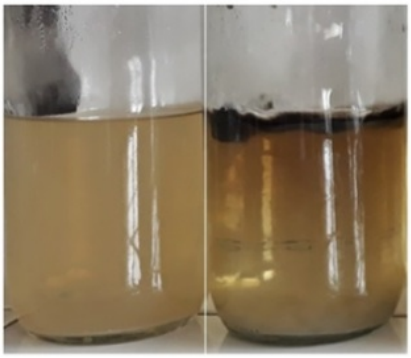

Figure 2. Control PDB (A); PDB after 30 days (B)

3.1 Detection of antibacterial potential isolate compounds

Based on Table 1 of twelve isolate compounds, two isolate compounds have active compounds against *Escherichia coli, Staphylococcus aureus, Shigella dysenteriae,* and *Salmonella typhi*, which are marked by the formation of clear zone area on the treatment medium namely DJS1 and DJS2. The biggest diameter inhibitory zone is seen in isolate DJS1

Table 1. Antibacterial Pontential Test Result of *Acanthus ilicifolius* Endophytic Fungi Isolates

Isolate	Diameter Inhibitory Zone(mm)			
	Escherichia coli	*Staphylococcus aureus*	*Salmonella typhi*	*Shigella dysenteriae*
AJS1		0	0	0
BJS1	0	0	0	0
BJS2	0	0	0	10.2
BJS3	0	10.8	0	13.7
BJS4	0	11.3	0	12.3
BJS5	0	0	0	0
DJS1	8.2	16.2	10.6	15
DJS2	16.7	10.7	13.3	12.9
DJS3	0	12.1	15.9	10.9
DJS4	0	0	0	0
DJS5	0	7.5	0	10.5
DJS6	11.6	11.2	8.7	6.9

which are 8.2 ; 16.2 ; 10.6 ; 15 mm and DJS2 which are 16.7; 10.7; 13.3; 12.9 mm. The isolates will be tested further to determine the minimum inhibitory concentration. The inhibitory zone formed on the test medium was due to the diffusion of active compounds from isolates on the paper disc to the medium, therefore isolates are tested that can inhibit the growth of the tested bacteria (Rosaline et al., 2016).

3.2 The Purification and Detection of Antibacterial Activity Compounds

Purification of secondary metabolites using column chromatography methods. The result of the purification of DJS1 secondary metabolite compound was obtained fourteen active eluates and DJS2 obtained thirteen active eluates. The fourteen eluates derived from DJS1 compounds, $DJS1_1$ eluates have antibacterial activity, while of thirteen eluates derived from DJS2 compounds, $DJS2_2$ elders, $DJS2_4$, and $DJS2_5$ have antibacterial activity. Each active eluent is then tested for antibacterial activity. The results of the antibacterial activity test can be seen in Table 2.

3.3 Bioautography Tests and Identification of Antibacterial Activity Compounds

Bioautography tests were performed on compounds with antibacterial activity to determine the Rf value. Different Rf values can indicate different compounds, different Rf values are influenced by the composition of the compounds used (Himanshu and Pradeep, 2012).

Each eluate has a different Rf value, except for $DJS1_1$ and $DJS2_2$ which have an Rf value of 0.58, but $DJS1_1$ and $DJS2_2$ come from different fungi isolates. $DJS2_4$ has an Rf value of 0.63 and $DJS2_5$ has an Rf value of 0.75. Based on Figure 3 and Table 3, the bioautography test results of compounds on $DJS1_1$, $DJS2_2$, $DJS2_4$, and $DJS2_5$ have the same inhibitory zone location with the chromatogram of each eluate compound. This indicates that the compound is active as an antibacterial because the compound from the TLC plate has diffused into the MHA medium after being allowed to stand for 1 hour (Kusumaningtyas et al., 2008; Choma and Grzelak, 2011).

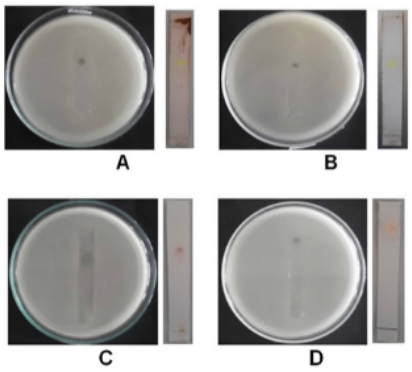

Figure 3. Bioautography test with $DJS1_1$ compound (A), $DJS2_2$ compound (B), $DJS2_4$ compound (C), and $DJS2_5$ compound (D)

After spraying with H_2SO_4 then heated on the hotplate, $DJS1_1$ and $DJS2_2$ eluates showed yellow spots. The yellow color in the $DJS1_1$ and $DJS2_2$ eluates are thought to be an active compound of the phenol group. The $DJS2_4$ compound eluate shows a purple spot, purple is a terpenoid compound (Salni et al., 2011). The $DJS2_5$ eluate showed an orange spot, orange is alkaloid compound (Paramita et al., 2018). The eluate compound Figure.4 shows one spot, one spot indicates that there is only one compound. That means the eluate compound is pure (Choma and Jesionek, 2015).

Phenolic compounds are bacteriocidal, phenol compounds

Table 2. Detection of antibacterial activity compound

Eluate	Diameter Inhibitory Zone (mm)			
	Escherichia Coli	*Staphylococcus auerus*	*Salmonella thypi*	*Shigella dysenteriae*
$DJS1_1$	7.1	12.5	6.9	19.7
$DJS1_2$	0	0	0	0
$DJS1_3$	0	0	0	0
$DJS1_4$	0	0	0	0
$DJS1_5$	0	0	0	0
$DJS1_6$	7.5	6.7	0	0
$DJS1_7$	0	6.8	0	0
$DJS1_8$	0	0	0	0
$DJS1_9$	6.7	0	0	0
$DJS1_{10}$	0	0	0	0
$DJS1_{11}$	0	0	0	0
$DJS1_{12}$	0	0	0	0
$DJS1_{13}$	0	0	0	0
$DJS1_{14}$	0	0	0	0
$DJS2_1$	6.7	0	8.8	0
$DJS2_2$	6.8	6.9	13.9	0
$DJS2_3$	7.8	0	0	0
$DJS2_4$	7.4	8.4	10.4	7
$DJS2_5$	0	9.9	10.8	6.8
$DJS2_6$	0	0	0	0
$DJS2_7$	0	0	0	0
$DJS2_8$	0	0	0	0
$DJS2_9$	6.7	0	0	0
$DJS2_{10}$	0	0	0	0
$DJS2_{11}$	0	0	0	0
$DJS2_{12}$	0	0	0	0
$DJS2_{13}$	0	0	0	0

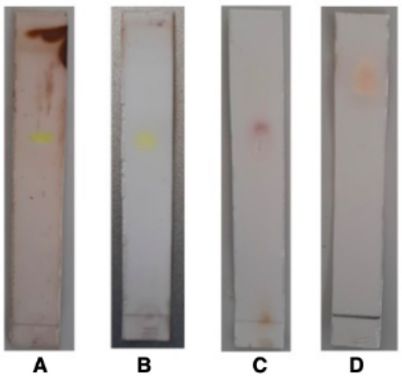

Figure 4. The result of identification eluates $DJS1_1$ (A), $DJS2_2$ (B),$DJS2_4$ (C), and $DJS2_5$ (D)

kill bacteria through the process of coagulation of proteins and damage cell membranes causing cell death (Septiani et al., 2017a). Terpenoid compounds can inhibit bacterial growth by interfering with the process of cell wall formation, cell walls are formed imperfectly (Jangnga et al., 2018). Alkaloid compounds can inhibit bacterial growth by damaging bacterial cell walls (Yuliana et al., 2017).

Table 3. Rf value and compound classes

Eluate	Rf	Color	Compound
$DJS1_1$	0.58	Yellow	Phenol
$DJS2_2$	0.58	Yellow	Phenol
$DJS2_4$	0.63	Purple	Terpenoids
$DJS2_5$	0.75	Orange	Alkaloids

3.4 Minimum Inhibitory Concentration (MIC)

The results of determining MIC eluates compound $DJS1_1$, $DJS2_2$, $DJS2_4$, and $DJS2_5$ can be seen in Table 4, Table 5, Table 6 and Table 7. It shows a decrease in the diameter

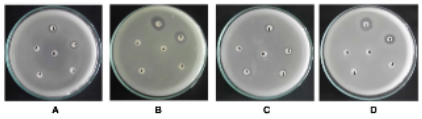

Figure 5. Determination of MIC value from $DJS1_1$, *Escherichia coli*(A), *Staphylococcus aureus*(B), *Salmonella typhi*(C), *Shigella dysenteriae*(D); 1000 ppm(1), 500 ppm(2), 250 ppm(3), 125 ppm(4), 62.5 ppm(5), 31.5 ppm(6)

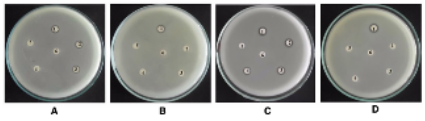

Figure 6. Determination of MIC value from $DJS2_2$, *Escherichia coli*(A), *Staphylococcus aureus*(B), *Salmonella typhi*(C), *Shigella dysenteriae*(D); 1000 ppm(1), 500 ppm(2), 250 ppm(3), 125 ppm(4), 62.5 ppm(5), 31.5 ppm(6)

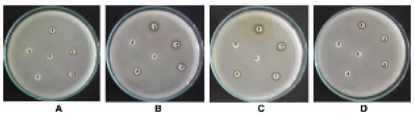

Figure 7. Determination of MIC value from $DJS2_4$, *Escherichia coli*(A), *Staphylococcus aureus*(B), *Salmonella typhi*(C), *Shigella dysenteriae*(D); 1000 ppm(1), 500 ppm(2), 250 ppm(3), 125 ppm(4), 62.5 ppm(5), 31.5 ppm(6)

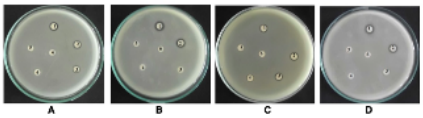

Figure 8. Determination of MIC value from $DJS2_5$, *Escherichia coli*(A), *Staphylococcus aureus*(B), *Salmonella typhi*(C), *Shigella dysenteriae*(D); 1000 ppm(1), 500 ppm(2), 250 ppm(3), 125 ppm(4), 62.5 ppm(5), 31.5 ppm(6)

of the inhibitory zone along with a decrease in the concentration of the compound. Determination of MIC values by dissolving each eluates using DMSO solvent with the following concentration ratio, 1000 ppm; 500 ppm; 250 ppm; 125 ppm; 62.5 ppm; 31.25 ppm. MIC test results can be classified into four categories namely, concentration <100 ppm is very strong, 100-500 ppm is strong enough, 500-1000 ppm is weak, and >1000 ppm is not having antibacterial activity (Holetz et al., 2002).

Based on Table 4 and Figure 5, the results of tests on the *Escherichia coli, Staphylococcus aureus,* and *Shigella dysenteriae* gave MIC values of 500 ppm, while *Salmonella typhi* MIC values at 250 ppm. The MIC of *Escherichia coli* at a concentration of 500 ppm was 6.9 mm, *Staphylococcus aureus* at a concentration of 500 ppm was 12.1 mm, and Shigella dysenteriae at a concentration of 500 ppm was 12.2 mm. *Salmonella typhi* at a concentration of 125 ppm was 6.4 mm, as shown in Figure 5. Based on the determination of MIC value from $DJS1_1$ eluate, the smallest active concentration that can still inhibit the growth of *Escherichia coli, Staphylococcus aureus,* and *Shigella dysenteriae* are at a concentration of 500 ppm, while Salmonella typhi is at a concentration of 125 ppm, the activity of this compound is quite strong.

Based on Table 5 and Figure 6, the results of tests on the *Staphylococcus aureus* and *Shigella dysenteriae* gave MIC values of 1000 ppm, while *Escherichia coli* gave MIC value at 250 ppm and *Salmonella typhi* MIC values at 125 ppm. The MIC of *Staphylococcus aureus* at a concentration of 1000 ppm was 7.2 mm, *Shigella dysenteriae* at a concentration of 1000 ppm was 10 mm, *Escherichia coli* at a concentration of 250 ppm was 6.8 mm, and *Salmonella typhi* at a concentration of 125 ppm was 6.8 mm, as shown in Figure 6. Based on the determination of MIC value from $DJS2_2$ eluate, the smallest active concentration that can still inhibit the growth of bacteria Staphylococcus and Shigella dysenteriae are at a concentration of 1000 ppm the activity of this compound is weak, Escherichia coli is at a concentration of 250 ppm and *Salmonella typhi* is at a concentration of 125 ppm, the activity of this compound is quite strong.

Based on Table 6 and Figure 7, the results of tests on the *Escherichia coli* and *Shigella dysenteriae* gave MIC values of 250 ppm, while *Staphylococcus aureus* and *Salmonella typhi* gave MIC value at 125 ppm. The MIC of *Escherichia coli* at a concentration of 250 ppm was 6.8 mm, *Shigella dysenteriae* at a concentration of 250 ppm was 10 mm, *Staphylococcus aureus* at a concentration of 125 ppm was 6.9 mm, and *Salmonella typhi* at a concentration of 125 ppm was 6.9 mm, as shown in Figure 7. Based on the determination of MIC value from $DJS2_4$ eluate, the smallest active concentration that can still inhibit the growth of bacteria *Escherichia coli* is at a concentration of 250 ppm, *Shigella dysenteriae* is at a concentration of 250 ppm, *Staphylococcus aureus* is at a concentration of 125 ppm, and *Salmonella typhi* is at a concentration of 125 ppm, the activity of this compound is quite strong.

Based on Table 7 and Figure 8, the results of tests on the *Staphylococcus aureus* and *Shigella dysenteriae* gave MIC values of 500 ppm, while *Escherichia coli* gave MIC value at 250 ppm and *Salmonella typhi* gave MIC value at 125 ppm. The MIC of *Staphylococcus aureus* at a concentration of 500 ppm was 9.9 mm, *Shigella dysenteriae* at a

Table 4. Determination of MIC value of $DJS1_1$

Eluate Compound	Concentration (ppm)	Diameter Inhibitory zone (mm) *Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	Shigella *dysenteriae*
$DJS1_1$	1000	7.3	16.8	7.7	16.5
	500	6.9	12.1	7.3	12.2
	250	0	0	6.7	0
	125	0	0	0	0
	62.5	0	0	0	0
	31.25	0	0	0	0

Table 5. Determination of MIC value of $DJS2_2$

Eluate Compound	Concentration (ppm)	Diameter Inhibitory zone (mm) *Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	Shigella *dysenteriae*
$DJS2_2$	1000	7.5	7.2	9.1	10
	500	7.2	0	8.8	0
	250	6.8	0	7.6	0
	125	0	0	6.8	0
	62.5	0	0	0	0
	31.25	0	0	0	0

concentration of 500 ppm was 8.9 mm, *Escherichia coli* at a concentration of 250 ppm was 6.9 mm, and *Salmonella typhi* at a concentration of 125 ppm was 6.7 mm, as shown in Figure 8. Based on the determination of MIC value from DJS eluate, the smallest active concentration that can still inhibit the growth of bacteria *Staphylococcus aureus* is at a concentration of 500 ppm, *Shigella dysenteriae* is at a concentration of 500 ppm, *Escherichia coli* is at a concentration of 250 ppm, and *Salmonella typhi* is at a concentration of 125 ppm, the activity of this compound is quite strong.

MIC test is a way to determine the sensitivity of a compound in inhibiting bacterial growth. The higher sensitivity of a bacteria indicates the lower the sensitivity of a compound (Maryadi et al., 2017). This research shows that the best MIC value is on the $DJS2_4$ eluate which can inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Shigella dysenteriae* in 250 ppm concentration. Based on the identification of $DJS2_4$ eluate containing terpenoids compound, terpenoids inhibit bacterial growth by forming reactions with transmembrane proteins on the outer membrane of bacterial cell walls to form very strong polymeric bonds and cause damage to transmembrane proteins. Damage to the transmembrane protein results in disruption of the permeability of the bacterial cell wall so that over time the bacteria will lack nutrients that disrupt bacterial growth and bacterial cell death (Salni et al., 2011).

3.5 Identification of the endophytic fungi

Endophytic fungi produce active compounds as antibacterials that are identified to determine the species of endophytic fungi. The identification results were carried out by the Genetics Lab Division, PT. Genetics Science Indonesia, West Jakarta, two against endophytic fungi isolates namely DJS1 and DJS2. DJS1 was identified as *Culvularia lunata* and DJS2 identified as *Diaporthe phaseolorum*, as seen in Figure 9.

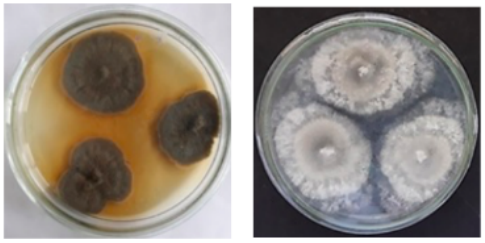

Figure 9. *Culvularia lunata*(A), *Diaporthe phaseolorum*(B)

4. CONCLUSIONS

This study shows the antibacterial activity of the secondary metabolites of the *Acanthus ilicifolius* endophytic fungi. *Culvularia lunata* and *Diaporthe phaseolorum* are endophytic fungi that have antibacterial activity. *Culvularia lunata*

Table 6. Determination of MIC value of $DJS2_4$

Eluate Compound	Concentration (ppm)	Diameter Inhibitory zone (mm) *Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	Shigella *dysenteriae*
$DJS2_2$	1000	7.2	11.6	11.3	7.9
	500	7	11.3	11	7.3
	250	6.8	8.3	7.9	7
	125	0	6.9	6.9	0
	62.5	0	0	0	0
	31.25	0	0	0	0

Table 7. Determination of MIC value of $DJS2_5$

Eluate Compound	Concentration (ppm)	Diameter Inhibitory zone (mm) *Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	Shigella *dysenteriae*
$DJS2_5$	1000	8	12.2	7.9	12
	500	7.7	9.9	7.6	8.9
	250	6.9	0	7.4	0
	125	0	0	6.7	0
	62.5	0	0	0	0
	31.25	0	0	0	0

is an endophytic fungi that produces active compounds as antibacterial, which is a phenol compound. *Diaporthe phaseolorum* is an endophytic fungi that can produce active compounds as an antibacterial which are phenol, terpenoid, and alkaloid.

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