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Active Compound Phenyl Ether from Potential Endophytic Fungi of *Syzygium zeylanicum*

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Abstract

Endophytic fungi can be found in various types of plants, especially medicinal plants, such as *Syzygium zeylanicum*, one of the many medicinal plants found in Indonesia. This study aimed to obtain isolates of endophytic fungi from the bark of *Syzygium zeylanicum*, L., with antibacterial and antioxidant activities and identify their bioactive compounds. Characterization of endophytic fungi was carried out morphologically and fungi that showed high bioactivity were characterized molecularly. Antibacterial activity was carried out by disc diffusion method and antioxidant activity by DPPH method. Isolation of bioactive compounds was carried out by chromatographic techniques and determination of the structure of pure chemical compounds by spectroscopic analysis. In this study, 3 isolates of endophytic fungi were obtained. Based on their morphological characterization, the endophytic fungi belong to the genera Trichoderma (Code SZT2 and SZT6), and Sclerotium (Code SZT8). Trichoderma mushroom extract (SZT2) gave high antibacterial activity (74.0 % against *E. coli*; 78.2 % against *S. aureus*; 80.5 % against *S. tyhpi* and 74.0 % against *B. subtilis*). The antioxidant activity was very strong SZT2 (IC_{50} = 5.83 µg/mL) SZT2 potential endophytic fungus based on molecular analysis is *Trichoderma koningi*. SZT2 mushroom extract has an active compound in the form of compound 3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-ɣ-butyrolactone based on the results of compound isolation using NMR and HMR. These endophytic fungal compounds can be developed into new sources of antibacterial and antioxidant bioactive compounds through a series of further studies.

Keywords: Active compound, Antibacterial, Antioxidant, Endophytic fungi, Phenyl compound

Introduction

The search for sources of bioactive compounds is continuously carried out in line with the increasing number of new diseases that appear, ranging from infectious diseases, cancer, and several other dangerous diseases. Bioactive compounds can be obtained from several sources, including from plants, animals, microorganisms. One source of bioactive compounds derived from microorganisms is endophytic fungi. The presence of endophytic fungi in plants can encourage them to produce compounds that act on phytopathogens or change plant morphology so that they are better able to defend themselves in unfavorable situations [1]. Endophytic fungi can produce bioactive compounds exclusively for their host plants, compounds can be the same or different from the host [2], which has the potential to be developed into drugs so that it has great potential in the search for new drug sources. This is because endophytic fungi are easy to grow, have a short life cycle and can produce large amounts of bioactive compounds by the cultivation method.

The use of antibiotics is the main driver of the emergence of antibiotic resistance [3], Antibiotic resistance is a global public health problem. The development of resistance by pathogenic bacteria to commercial drugs is a relevant problem faced by health services and has become a serious concern worldwide. Some of the factors that cause this resistance are the extensive and often inappropriate use of antibiotics, poor hygienic conditions, the constant movement of tourists, the increasing number of patients with compromised immune systems, and delays in the diagnosis of infections. As a result, there is a need

for intensive search for new antimicrobial agents from various natural sources including endophytic fungi. Endophytic fungi internally colonize plant tissues and have the potential to act as control agents, such as biologic agents or elicitors in resistance induction processes and in attenuating abiotic stresses [1]. The potential of the *Syzygium zeylanicum* plant as an antibacterial was described in the study of [4] here the leaf extract had a Minimum Inhibitory Concentration (MIC) value of 350 g/mL for *E.coli* and and *P. aeruginosa*, 1,000 g/mL for *P. pneumonia* and 500 g/mL for *B. subtilis*.

Free radicals are reactive oxygen and nitrogen that go through various physiological processes in the human body [5]. In addition, the effects of free radicals in the body that can interfere with cell integrity, can react with cell structural components such as molecules that make up membranes and functional components such as enzymes and DNA. Free radicals in the body can cause various degenerative diseases such as cancer, atherosclerosis, rheumatoid, arthritis, diabetes, decreased immune response to the aging process. To protect the body from the effects of free radicals, a vital substance is needed, namely antioxidants. Research [6], found compound zelaniin A compounds in the leaves of S. zeylanicum which showed strong antioxidant activity of 19.18 ± 0.24 and 4.37 ± 0.29 (mol TE/mol) in the DPPH and ORAC tests, and lipid peroxidation inhibition was almost 80 % at the level of 100 g/mL (78.76 ± 0.21 %).

Endophytic fungi, especially those that live in medicinal plant tissues, are often used as a source for the discovery of bioactive compounds. Some plants can degrade the bioactive compounds they contain to endophytic microbes that grow in their tissues, so that these endophytic microbes can produce the same compounds as the hosts. In our previous study [7] found antibacterial compound p-hydroxy benzaldehyde from endophytic fungi isolated from the root bark of *Syzygium zeylanicum* is p-hydroxy benzaldehyde compound is also produced by its host. To continue our series of research, in this paper we report the bioactive potential of endophytic fungi from other parts of the *S. zeylanicum* plant, namely the leaves and bark. This *Syzygium zeylanicum* plant has been used by people in Indonesia as a medicinal plant related to pathogenic bacterial infections and the effects of free radicals in the body.

Materials and methods

Plant material

Endophytic fungi were isolated from the bark of *S. zeylanicum* (L.) from Penukal Abab Lematang Ilir (Pali) Regency, South Sumatra. Plants have been identified in the laboratory of the LIPI Biology Research Center in the field of botany. Sampling was carried out in a fresh state in November 2020. Endophytic fungi were isolated from the bark organ. The bark used is the outermost bark of the main stem.

Isolation of endophytic fungi

Isolation of the endophytic fungi was started by sterilizing the surface of the *Syzygium zeylanicum* plant stem bark and rice. Fresh plant organs were washed with running water until clean for ± 5 min. Then soaked in 70 % alcohol for ± 3 min. Then rinsed with sterile distilled water for ± 1 min, then soaked with Sodium hypochloride (NaOCl) 3 % (w/v) for 1 min. The bark is cut by ± 3×0.5 cm^2. Then the samples were planted in petri dishes containing PDA media, incubated at room temperature for 3 - 14 days. Observations were made every day until the fungus was visible. Fungal colonies growing on PDA medium that showed different morphological properties (shape, color and size) were further purified. Purification was carried out by transferring the colonies to a new PDA medium with a single spore isolation method, then incubating at room temperature for 2×24 h. The separate fungal colonies were purified and then made a working culture (in Petri dishes) and stock cultures (in test tubes) by growing on PDA media [8]–[10].

Identification of endophytic fungi

Identification of endophytic fungi was carried out based on their phenotypic characters macroscopically and microscopically. Observations of colony characteristics include; a) colony color and reverse colony color (reverse side), b) surface of the colony; granular, like flour, ridged, smooth, c) presence or absence of drops of exudate, d) presence or absence of radial lines (radial furrow) from the center of the periphery of the colony, e) presence or absence of circles-concentric circle. And microscopic observations include the shape of the hyphae or mycelium, the shape of the spores, the color of the spores, the presence or absence of a septum on the hyphae and other microscopic characteristics. The phenotypic identification data was then compared with identification key books such as identification keys Pictorial

Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species [11], Larone's medically important fungi [12], and Fungi and Food Spoilage [13].

Molecular analysis of ITS rDNA

Isolates were identified based on the Internal Transcribed Spacer (ITS) area of DNA (rDNA). Amplification using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') [14]. DNA sequences of forward and reverse primers were compiled using the Bioedit program. The sequence results were then identified to the level of taxa species using the online bioinformatics method of the Basic Local Alignment Search Tool (BLAST) at the website address http://blast.ncbi.nlm.nih.gov/Blast.cgi. Next, the sequences were multiple aligned using the MEGA11 program with the CLUTAL W method and a phylogenetic tree was constructed using the Neighbor-joining tree method with a bootstrap value of 1,000 [15], [16].

Cultivation and extraction

All endophytic fungal strains were cultivated by placing 6 blocks of pure culture agar (± 6 mm in diameter) into 300 mL Potato Dextrose Broth (PDB) agar medium. Each isolate was cultivated as much as 300 mL ×5 Erlenmeyer flasks. The cultures were then incubated for 4 weeks at room temperature under static conditions. After the incubation period, the mycelia were separated from the broth culture using filter paper. Then ethyl acetate solvent was added to the culture medium (1:1) and extracted by partition (repeated 3 times). The ethyl acetate extract was separated from the liquid culture and evaporated using a rotary evaporator to obtain a clear extract [4], [9], [17]. The extract was thickened using an oven at 40 °C. The viscous extract and biomass were weighed using an analytical balance.

Antibacterial activity test

Antibacterial activity testing Kirby-Bauer method with NA (Sodium Agar) medium. There were 4 test bacteria used, namely 2 g negative bacteria (*Escherichia coli* InaCCB5 and *Salmonella thypi* ATCC1048 and 2 g positive bacteria (*Staphylococcus aureus* InaCCB4 and *Bacillus subtilis* InaCCB1204). The blank disc paper was dripped with 20 µL endophytic fungal extract with a concentration of 400 µg/disk. Dilution of the mushroom extract using dimethylsulfoxide (DMSO), then left until all the solvent evaporates completely. Positive control uses Tetracycline 30 µg/disk. The disc paper containing the test solution is placed on the surface of the media that has been inoculated with the test bacteria. Then it is incubated for 1×24 h at room temperature. 37 °C, then observed the inhibition zone. The diameter of the formed inhibition zone was measured using a caliper. Determination of the antibacterial activity of the test sample and the criteria for the diameter of the inhibition zone were determined by the following equation:

Weak : $\frac{A}{B}$x 100 % < 50 %; Currently: 50 % <$\frac{A}{B}$x 100 % < 70 %; Strong: $\frac{A}{B}$x 100 % > 70 %

A: Zone of inhibition (mm) test sample, and B: Zone of inhibition (mm) of standard antibiotics.

Antioxidant activity test

Antioxidant activity was determined using the DPPH method. The fractions obtained from the extraction procedure were dissolved into concentrations of 1,000, 500, 250, 125, 62.5, 31.25, 15.625 g/mL. 0.2 mL of each concentration was added 3.8 mL of 0.5 mM DPPH. As a standard antioxidant ascorbic acid was used and at least 3 replicates of each concentration were considered [18]. The mixture was homogenized and left in a dark tube for 30 min. Absorption was measured using a UVVis spectrophotometer at max 517 nm. In this test, ascorbic acid was used as a standard positive control and methanol as a negative control. Antioxidant activity can be represented by the value of DPPH absorption inhibition, which is calculated by the percentage inhibition of DPPH absorption and the IC50 value [17]:

$$\% \text{ Inhibition} = \frac{A_k - A_s}{A_s}$$

A_k = Absorbance of control, and A_s = Absorbance of samples.

Isolation and identification of bioactive compound

The crude extract which had strong antibacterial activity was separated using column chromatography (Stationary phase: Silica gel) and eluted with graded eluent (N-hexane: Ethyl acetate: Methanol). Drops of eluate were collected every 10 mL in vials and monitored by TLC analysis to group vials into subfractions. The subfraction identified as having secondary metabolites was then purified column chromatography until pure compounds were obtained. These structures were identified by spectroscopic methods including 1H-NMR, 13C-NMR, HMQC, HMBC [17].

Results and discussion

lation of endophytic fungi

The results of the isolation of endophytic fungi from the bark obtained 3 isolates with codes SZT2, SZT6, and SZT8. These fungal colonies showed different morphological properties (shape, color, and size) and were further purified. The distribution of the mycelium is dominated by a widespread white color (**Figure 1**).

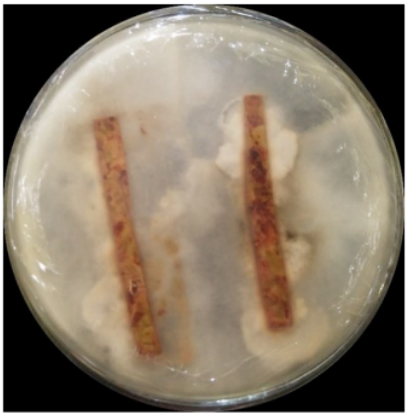


Figure 1 Mycelium growth of endophytic fungi from stem organs of *Syzygium zeylanicum*.

SZT2 colonies were characterized by greenish white in front with light yellow on the contrary, powdery texture, flat surface type and diffuse growth pattern (**Table 1**). SZT6 has a characteristic green color with white edges and a yellow opposite color with a zoning and spreading pattern that has a cotton-like structure (**Table 1**) and ZT8 has a characteristic white color with the same opposite color as white with a cotton-like structure with a zoning pattern (**Table 1**).

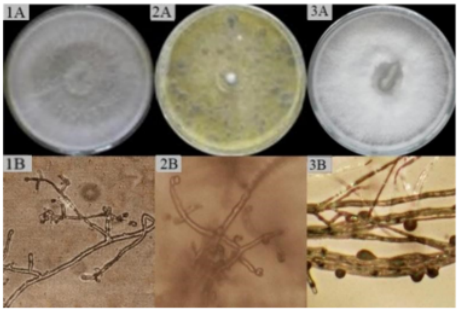


Figure 2 Conidial and hyphal morphology of endophytic fungal species; SZT2 (1); SZT6 (2); SZT8 (3S) fungal colony (A); Fungal spore (B).

Phenotypic analysis showed that the 3 isolates wer rouped into 2 classes, namely Sordariomycetes SZT2 and SZT6), and Deuteromycetes (SZT8) **Table 1**. Endophytic fungi isolated from the bark of *S.*

zeylanicum showed varied spore forms (verticillate and globose). Microscopic characteristics of isolates ZT2 and ZT6 have conidia spore type with round, erect, branched vertically arranged shape (**Table 1**), there is a difference in the irregular position of ZT2 phialid while ZT6 has short and thick phyalid so that ZT2 isolate is close to *Trichoderma koningi* species. and isolate SZT6 identified *Trichoderma pseudokoningi* [11]. Characteristics of isolates ZT8 has a type of conidia spore with a round shape that has septate hyphae with a specific character of hyphae branching very close and narrow at the base, there is a clamp connection (**Table 1**) so that the isolate ZT8 was identified as *Sclerotium complanatum* [11]. Morphological identification has limitations on incomplete observations in 1 fungal cycle, so it needs to be strengthened by molecular identification in the ITS rDNA area.

Table 1 Colony characteristics of endophytic fungi from the Stem bark of *S. zeylanicum*.

	Decription	SZT2	SZT6	SZT8
Macroscopic	Colony	Greenish white	Grayish with dark green spot	Milky white
	Reverse colony	Light yellow	Light yellow	White
	Structure	Powdery	Powdery	Cottony
	Elevation	Flat	Flat	Flat
	Pattern	Spread	Spread	Zonate
	Exudate drop	No	No	No
	Radial Line	No	No	Yes
	Concentric line	Yes	Yes	No
Microscopic	Spore	Conidia	Conidia	Conidia
	Shape	Globose	Globose	Globose
	Hyphae	Septate	Septate	Septate
	Characteristic	Phialides located irregular	Phialids short and thick. Bearing spore masses apical	Side branched septate very closely near main hyphae
	Genus/Species	*Trichoderma harzianum*	*Trichoderma pseudokingi*	*Sclerotium complanatum*

Note: (−) = characteristic doesn't appear; (√) = characteristic appear

Antioxidant and antibacterial activit

Four bacterial pathogens, including *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* were used for the analysis of antibacterial activity by the KirbyBauer method. The antibacterial and antioxidant activity of the ethyl acetate extract of the endophytic fungus *S. zeylanicum* are shown in **Tables 2** and **3**. Isolates with strong antibacterial activity on the 4 pathogenic microbes were shown by isolate SZT2. Followed by isolate SZT6 which has moderate antibacterial activity against 4 bacteria. And isolate SZT8 showed weak antibacterial activity on *B. subtilis* and *E. coli*.

Table 2 Antibacterial activity of endophytic fungus *S. zeylanicum*.

Isolate code	Genus/Species of Identification	Ethylac etate extract weight (gram)	% Antibacterial activity			
			E. coli	*S. aureus*	*S. thypi*	*B. subtilis*
SZT2	*Trichoderma koningi*	4.8	74.0 ± 0.81***	78.2 ± 1.72***	80.5 ± 0.74***	74.0 ± 4.10***
SZT6	*Trichoderma pseudokingi*	5.8	65.1 ± 0.49**	66.7 ± 0.23**	61.0 ± 0.52**	54.2 ± 0.39**
SZT8	*Sclerotium complanatum*	4.9	47.8 ± 0.17**	55.6 ± 0.18**	50.9 ± 0.39**	47.1 ± 0.35*
Positive control			Tetracycline 30 µg/disc			
			100***	100***	100***	100***

Note: Antibacterial activity percentage: *** = strong (≥ 70 %), ** moderate (50 - 70 %), and weak (< 50 %)

Table 3 Antioxidant activity of endophytic fungus *S. zeylanicum*.

Isolate code	Genus/Species of identification	Antioxidant Activity IC_{50} (µg/mL)	Category of Antioxidant Activity
SZT2	*Trichoderma koningi*	5.83****	Very strong
SZT6	*Trichoderma pseudokingi*	49.93***	Strong
SZT8	*Sclerotium complanatum*	18.03***	Strong
Positive control		Ascorbic Acid (µg/mL)	
		2.73****	Very Strong

Note: Antioxidant activity IC50 (µg/mL): **** very strong (≤ 10 µg/mL), *** strong (< 100 µg/mL) **moderate (100 - 500 µg/mL), *low (> 500 µg/mL)

Molecular identification of selected endophytic fungi

Molecular characterization combined with morphological identification can help mold taxonomy in differentiation to the species level [19]. Molecular characterization was carried out on the rDNA Internal Transcribed Spacer (ITS) area. Currently there are more than 90,000 fungal sequences in the ITS area, where this area is most widely used as a barcode area for fungi [20]. The results of the PCR product used a pair of universal primers, namely ITS1 for the forward primer and ITS4 for the reverse primer. The results of the amplification of the ITS rDNA region varied by ± 500 bp (**Figure 3**). Sequence readings from each of the forward and reverse primers were processed by cutting the ends of the sequences with low peaks using the Bioedit program. Furthermore, the sequences are straightened so that the compilation sequence of the forward and reverse primers from the ITS rDNA area is obtained.

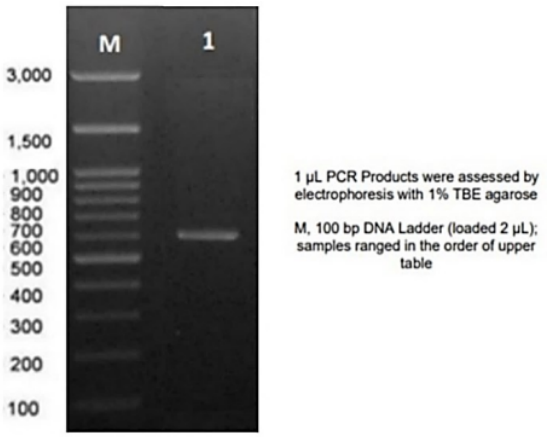


Figure 3 Electrophoresis results of ITS rDNA sequences of endophytic fungi.

Sequences of the ITS rDNA area of ZT1 ZT2 isolates were analyzed for intraspecies similarity with the data in the NCBI Genbank using the BLAST algorithm. The relationship between sequences can be observed through the construction of a phylogenetic tree using the distance calculation method, namely a phylogenetic tree that joins neighbors. The purpose of using this method is to determine the position of the isolate sequence to the nearest sequence (sequences that have small base pair differences) so that the distance between sequences can be known [21]. Sequence collections were aligned using CLUSTAL W in the MEGA11 program and a phylogenetic tree was constructed (**Figure 4**).

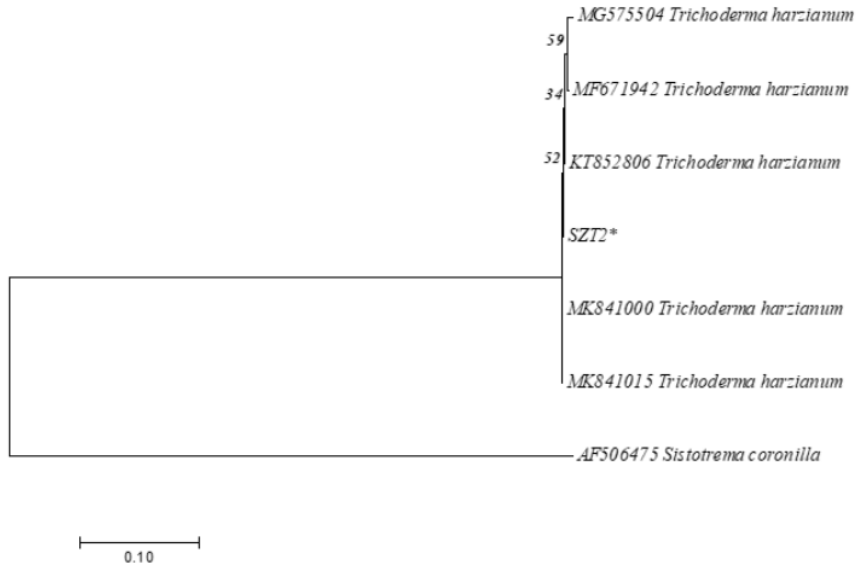


Figure 4 Phylogenetic tree construction of endophytic fungus SZT2.

Its construction uses the Neighbor-Joining method. The endophytic fungus *S. zeylanicum* marked with an asterisk (*) was subjected to phylogenetic analysis with related species using a joined neighboring phylogenetic tree (bootstrap value = 1,000). The sequence obtained from the BLAST results. The value in the branch shows the bootstrap value (percentage of 1,000×replications). The results of the phylogenetic tree construction of the endophytic fungi SZT2 are shown in **Figure 4**. The phylogenetic tree of SZT2 shows that the isolate sequence is close to *Trichoderma harzianum.*

Determination of chemical structure

Isolation of bioactive compounds was carried out on selected ethyl acetate extracts with the highest antibacterial and antioxidant activity, namely isolates SZT2. Concentrated ethyl acetate extract (2.0 g) was separated by gravity column chromatography (CC) method with a gradient eluent system, namely 100 % n-hexane (100 mL) eluent, a mixture of n-hexane and ethyl acetate with increasing polarity, (N-hexane: Ethyl acetate (100 mL), 8:2 (100 mL), 7:3 (100 mL), 5:5 (100 mL); 2;8 (100 mL), 100 % ethyl acetate (100 mL), and ethyl acetate and methanol (9:1 30 mL), 8:2 (30 mL), 7:3 (30 mL). The stationary phase used was silica gel 60 G (70 - 230 mesh). The separation results were collected using vials and obtained as many as 80 vials. The eluate was then analyzed using thin layer chromatography (TLC) with a mixed eluent of n-hexane and ethyl acetate (5:5). TLC with similar chromatogram patterns were combined into 1 fraction. Based on the results of the chromatogram pattern obtained 4 fractions. Namely F1 - F4. In the F2 fraction, white crystals were formed and after being purified with n-hexane.

The ^{1}H-NMR spectrum of compound A (**Figure 5**) showed the presence of 8 proton signals including 2 aromatic dublet signals with integration of 2 protons and an ortho-slotting constant (J = 9.0 Hz). This indicates that compound 1 is a para-substituted aromatic compound, so it has 2 pairs of equivalent protons. In addition there is a broad signal at δ_H 7.53 indicating a hydroxyl proton and 5 signals at the chemical shift δ_H < 6.5 ppm namely at δ_H 6.34, 5.30, 4.14, 3.78 and 3.67 ppm proton methine and proton methine oxygenated. Thus compound 1 was identified as a para-substituted aromatic compound having a hydroxyl group and oxygenated methine.

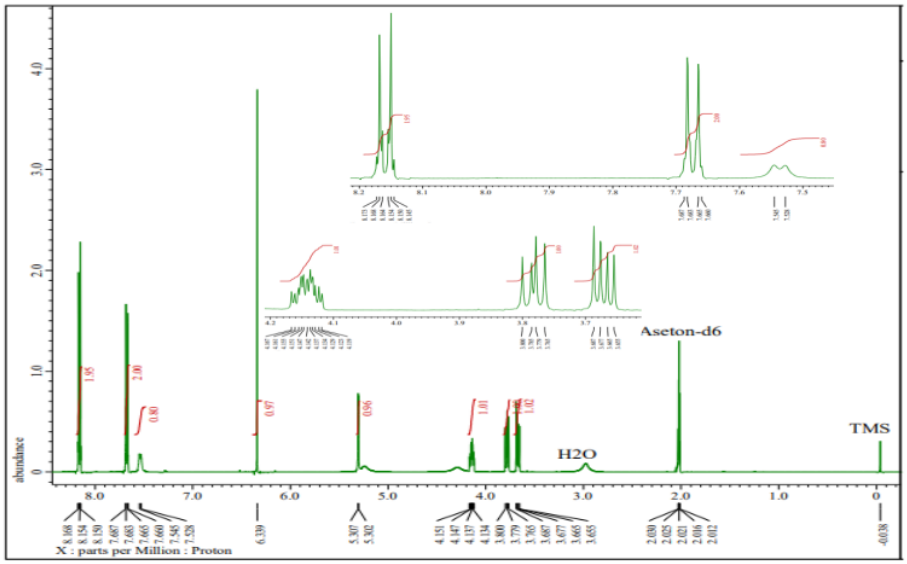
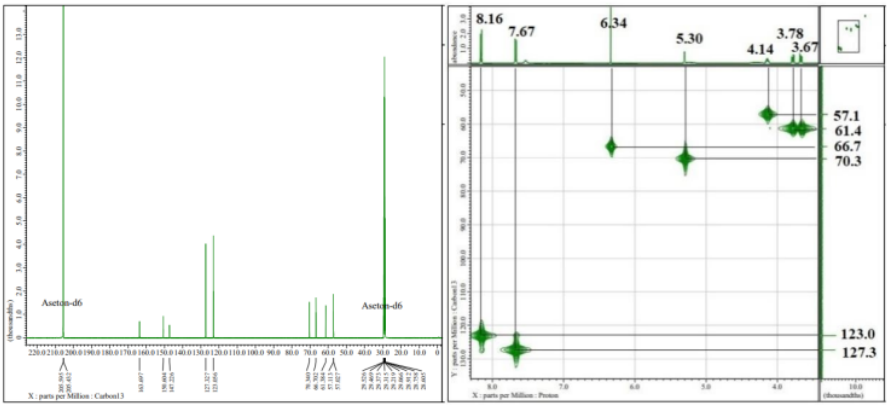


Figure 5 The [1]H-NMR spectra of compound B from endophytic fungi SZT2 ([1]H-500 MHz in aceton).

The ^{13}C-NMR spectrum of compound A (**Figure 6(A)**) showed the presence of 9 carbon signals. There are 2 high-intensity carbon signals indicating the presence of 2 pairs of equivalent aromatic carbons. Two other aromatic carbons as quaternary carbons appear with low intensity, namely at δ_C 147.2 and 150.6 ppm. Another carbon signal in the lowest field at δ_C 163.6 ppm is the carbonyl ester carbon atom. Three carbon signals at δ_C 60.0 - 71.0 ppm and 1 carbon signal at δ_C 57.0 ppm, each is a methine carbon signal, 3 of which are oxygenated methine carbon. The analysis of the ^{13}C-NMR spectrum was corroborated by the data on the HMQC spectrum (**Figure 10(B)**). The HMQC spectrum shows 7 ^{1}H-^{13}C correlations through 1 bond. Proton signals at δ_H 3.78 (1H, m) and 3.67 ppm (1H, m) showed a correlation to the same carbon atom at δ_C 61.4 ppm indicating a cyclic methylene group. Thus, compound B in addition to having a substituted benzene ring, also has a lactone ring with a methylene group on the ring.



(A) (B)

Figure 6 The ^{13}C-NMR (A) and HMQC (B) spectra of compound A ([1]H-500 MHz; ^{13}C-125 MHz in aceton.

The HMBC spectrum (**Figure 11**) showed a ^{1}H-^{13}C correlation through 2 or 3 bonds. The aromatic proton signal at δ_H 8.16 ppm correlates with 3 aromatic carbon atoms at δ_C 123.0, 147.2 and 150.6 ppm, including the carbon atom equivalent. The aromatic proton at δ_H 7.67 ppm is also correlated with 3 aromatic carbon atoms, namely at δ_C 123.0, 127.3 and 147.2 ppm and oxygenated carbon at 70.3 ppm C as aromatic ring substituents. The oxygenated methine proton at δ_H 5.30 ppm correlates with the 2 aromatic carbons at 127.3 and 150.6 ppm and with the 2 methine carbons 57.1 and 61. ppm. The correlation indicates that the oxygenated methine group is directly attached to the aromatic ring and is para substituted with a hydroxyl group. Furthermore, the correlation of the 2 methylene protons, namely δ_H 3.67 (1H, dd, J = 10.5 and 5.0 Hz) and 3.78 (1H, dd, J = 10.5 and 7.5 Hz) to the same carbon atom, is at δ_C 57.1 and 70.3 ppm. The difference in the value of the plotting constant of the 2 methylene protons proves that these 2 protons are not equivalent. The spectrum also shows the correlation of the oxygenated proton at δ_H 6.34 ppm and the hydroxyl proton at δ_H 7.53 ppm to the carbonyl ester carbon atom δ_C 167.3 ppm through 2 and 3 bonds. The 1D and 2D NMR spectral data for compound 1 are shown in **Table 4**.

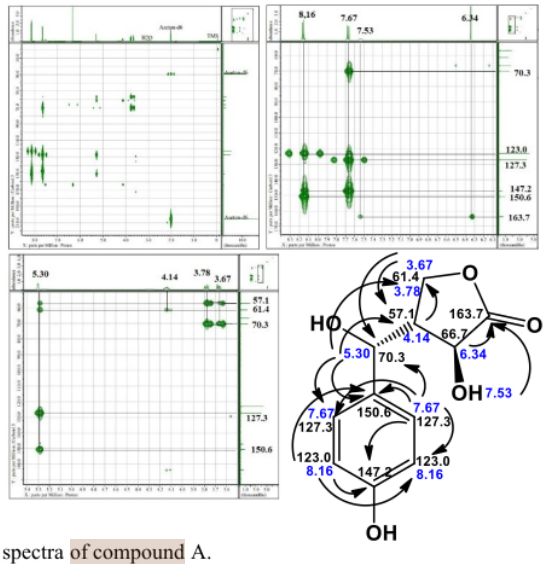


Figure 7 The HMBC spectra of compound A.

Table 4 The NMR data of compound A, recorded at ^{1}H-500 MHz; ^{13}C-125 MHz in aceton.

No. C	δ_C ppm	Type of C	δ_H ppm (ΣH. multiplicity. *J* (Hz))	HMBC
2	163.7	C		
3	66.7	CH	6.34 (1H, s)	163.7
4	57.1	CH	4.14 (1H, m)	61.4
5	61.4	CH_2	A. 3.67 (1H, dd, J = 10.5 and 5.0 Hz) B. 3.78 (1H, dd, J = 10.5 and 7.5 Hz)	57.1, 70.3
6	70.3	CH	5.30 (1H, d, J = 2.5 Hz)	57.1, 61.4, 127.3, 150.6
1'	150.6	C		
2'	127.3	CH	7.67 (1H, d, J = 9.0 Hz)	70.3, 123.0, 127.3, 147.2
3'	123.0	CH	8.16 (1H, d, J = 9.0 Hz)	123.0, 147.2, 150.6
4'	147.2	C		
5'	123.0	CH	8.16 (1H, d, J = 9.0 Hz)	123.0, 147.2, 150.6
6'	127.3	CH	7.67 (1H, d, J = 9.0 Hz)	70.3, 123.0, 127.3, 147.2
3-OH			7.53 (1H, d, J = 7.5 Hz)	163.7

Based on spectral analysis of ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC, it can be explained that compound A has a para-substituted benzene ring between the hydroxyl group and the oxygenated methine group. This oxygenated methine group binds to the 3-hydroxy-ɣ-butyrolactone ring. Thus, the proposed chemical structure of compound A is 3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-ɣ-butyrolactone as shown in **Figure 8**.

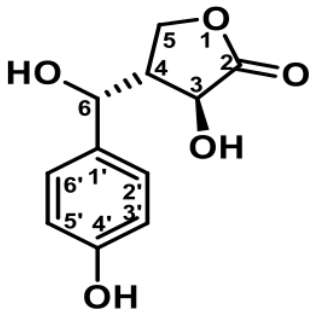


Figure 8 The structure of compound A as 3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-ɣ-butyrolactone.

Effect of compound structure on antibacterial and antioxidant activity

From the results of the bark isolation, a compounds were identified, ZT2 (3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-ɣ-butyrolactone). A compounds have good activity against antibacterial and antioxidant. The compound SZT2 (3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-ɣ-butyrolactone) has 1 hydroxyl-substituted aromatic ring (−OH) on the ring by binding another hydroxyl to the alkyl substituted at the para (p) position in the ring. benzene ring. The SZT2 compound, when viewed from its structure, belongs to the secondary metabolite compound of the phenolic group so that it has a similar mechanism of action to compounds with other groups of the same group in this group, which can cause tissue shrinkage due to complexation with microbial substrates enzymes through hydrogen bonding with bacterial proteins. The H+ ion from the ZT2 compound will attack the polar group (phosphate group) so that the phospholipid molecules will break down into glycerol, carboxylic acid, and phosphoric acid [22]. Hydrogen bonds med between phenol and protein cause damage to the protein structure. Hydrogen b s will affect the permeability of cell walls and cell membranes because they are composed of proteins. Permeability of cell walls and cell membranes that are disrupted can cause an imbalance of macromole s and ions in the cell, so that the cell becomes lysed [22].

From **Table 2** the antioxidant activity for the compound ZT2 is IC_{50} = 5.83 (µg/m The smaller the IC_{50} value, the better the antioxidant activity given. From the data, it can be seen that the antioxidant activity of the ZT2 compound is the best, this can be seen from the structure of the ZT2 compound which has more substituted hydroxyl groups (−OH) compared to other compounds. In addition to the hydroxyl group, other functional groups that have lone pairs of electrons, are heteroatoms and act as schiff bases also have the ability to donate electrons so as to stabilize the structure by experiencing resonance with the benzene nucleus. The more hydroxyl groups bound to phenolic compounds will increase the ability of these compounds to donate protons to DPPH free radicals, thereby increasing the potential for antioxidant activity [23].

The new natural product 4'-hydroxy-deacetyl-18-deoxycytochalasin H, together with the known deacetyl-18-deoxycytoch in H and 18-deoxycytochalasin H were obtained from the endophytic fungus Trichoderma harzianum iso d from leaves of Cola nitida. The structure of the new compound was unambiguously determined by 1D an D NMR spectroscopy, and by HRESIMS measurements, as well as by comparison with the literatur Compounds 1-3 showed potent cytotoxic activity against the murine lymphoma (L5178Y) cell line and against human ovarian cancer (A2780 sens and A2780 CisR) cell lines (IC50 0.19 - 6.97 µM). The A2780 cell lines included cisplatin-sensitive (sens) and -resistant (R) cells [24].

Conclusions

Endophytic fungi have been successfully cultured and 4 fungal isolates were obtained from the bark (with codes SZT2, SZT6, and SZT8). Based on morphological characterization, the endophytic fungi were included in the genus Trichoderma and Sclerotium. All endophytic fungi have potential antibacterial and antioxidant activity. The molecular results of the isolate with the strongest antibacterial against 4 pathogenic bacteria showed that the isolate SZT2 was identified as *Trichoderma harzianum*. Based on the results of the isolation of bioactive compounds, new bioactive compound was identified, namely 3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-ɣ-butyrolactone from isolate ZT2, respectively. These endophytic fungal compounds can be developed into new sources of antibacterial and antioxidant bioactive compounds through a series of further studies.

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