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Antioxidant and Antibacterial Activity of Endophytic Fungi Isolated from The Leaves of Sungkai (*Peronema canescens*)

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ABSTRACT

Sungkai (*Peronema canescens*) is a medicinal plant widely used in Indonesia. Its leaves are believed to have the potential to treat fever and boost the immune system. This study investigated the antioxidant and antibacterial activity of endophytic fungi extracts obtained from Sungkai leaves. This will provide basic information for developing the potential of natural ingredients as antioxidants and antibacterials. Endophytic fungi residing in Sungkai leaves were identified morphologically. The antioxidant test was completed using DPPH, while the antibacterial activity was tested using the paper disk diffusion method. Most potential endophytic fungal isolates were identified through molecular identification, and the isolation of a bioactive compound was achieved using column chromatography. The structure of the compound was determined using 1D and 2D NMR spectroscopy methods. Four endophytic fungi isolates were observed in the Sungkai leaves (code RND1–RND4). RND3 demonstrated very strong antioxidant activity (IC_{50} < 20 µg/mL) and strong antibacterial activity. Based on the molecular test, RND3 was identified as *Aspergillus niger*. The pure compound isolated from RND3 revealed weak and strong antioxidant (IC_{50} > 500 µg/mL) and antibacterial (MIC ≤ 64 µg/mL) properties, respectively. Spectroscopic analysis showed that compound 1 was 3-benzyl-2,6-dihydroxy-1,4,11,13-tetramethyl-5-methylene-12,15-dioxo-14-oxabicycloheptadeca-8,16-diene-7-carboxylic acid. Through further research, this study can be used as a basis for the development of compounds as raw materials for drugs.

Keywords: Antibacterial, Antioxidant, Endophytic Fungi, Sungkai

Introduction

Various species of medicinal plants with diverse healing properties are often used by humans. Undiscovered drugs are likely present in plants that have not yet been investigated.[1–3] Currently, it is estimated that more than 50,000 species of medicinal plants are used worldwide. This is the largest spectrum of biodiversity used by humans within a particular period of time.[4–6] In Indonesia, the use of medicinal plants is widely practiced in both rural and urban areas. The COVID-19 pandemic generated concern among the public, driving the belief that medicinal plants can prevent and even cure various kinds of infectious diseases, including COVID-19. In particular, Sungkai (*Peronema canescens*) has been used by society as a plant that can treat COVID-19.[7–9]

Sungkai has the properties of treating fever, flu, and cough, so it is often processed into simple concoctions for use as a traditional medicine.

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The plant, also known as Jati Sebrang or Sekai, contains many secondary metabolites, such as alkaloids, sitosterol, saponins, tannins, terpenoids, diterpenoids, and flavonoids.[10–13] Secondary metabolites from Sungkai have been reported to have antibacterial, antioxidant, antimalarial, and antidiabetic activities and can reduce uric acid levels.[14–20] The betulinic acid and peronemin in Sungkai have also been reported as antioxidants and antibacterials. The antioxidant and antibacterial are known to boost the immune system.[21,22]

Antioxidants are very effective in counteracting free radicals in the body. Free radicals are known to be the main cause of diseases such as cancer, hypertension, and degenerative disorders.[23–25] The use of synthetic antioxidants has been reported to trigger mutagenic effects, so natural ingredients are necessary to avoid various kinds of disturbances in the body.[26–29] Apart from synthetic antioxidants, bacterial resistance has also become the focus of current research. Existing antibiotics possess various disadvantages, including reactions that cause hypersensitivity.[30,31] Bacterial resistance and the negative effects of synthetic materials make the use of drug sources in pharmaceuticals indispensable. Thus, the utilization of ingredients from plants has increased, which has caused a depletion of natural resources. However, endophytic fungi can be an alternative, making them the main focus of the current research.

Endophytic fungi are fungal colonies that are symbiotic with plant tissues. The existence of these interactions causes endophytic fungi to be able to copy and even modify secondary metabolites from their host plants.[32–36] The secondary metabolites of these endophytic fungi are believed to be the most relevant discoveries for new medicinal materials.[37–39] They have the potential to overcome the synthetic antibiotics and antioxidants that have been used until today. Studies have revealed that most of the compounds produced by endophytic fungi isolated from medicinal plants have a distinctive structure and the same or even better bioactivity compared to their hosts.[40–42] Therefore, this study aimed to explore new compounds with antioxidant and antibacterial activity obtained from various types of endophytic fungi isolated from the Sungkai plant.

Materials and Methods

Preparation of plant samples

Sungkai were collected from Ogan Ilir Regency, South Sumatra. The old leaves (dark green, healthy, and located in the second position from the base of the branch) were taken fresh in March 2021. The plant sample was identified in the laboratory of Biosystematic, University of Sriwijaya, with number 302/UN9.1.7/4/EP/2021.

Isolation of Endophytic Fungi

The fresh leaves are washed with water for ± 4 minutes. Then, the leaves were immersed in sodium hypochlorite (NaOCl) for ± 1 minute, soaked for ± 1 minute in 70% alcohol, and rinsed with sterile distilled water for ± 1 minute. After the surface was sterilized, the leaves were bruised aseptically and inoculated into a petri dish containing Potato Dextrose Agar (PDA) media. Next, the samples were incubated for 5-14 days at room temperature. Fungal colonies growing around leaves with different characteristics were then purified into new petri dishes containing PDA media and incubated for 2-5 days at room conditions.[43]

Identification of Endophytic Fungi Morphologically

The macroscopic and microscopic characteristics of the endophytic fungi isolates were used for the identification process. Macroscopic characteristics include colony surface color, reverse color of the colony, colony texture, appearance of exudate dots, radial lines, and concentric circles while microscopic characteristics include hyphae (septae or not) and spores which are observed through culture slides under a microscope up to 1000X magnification. These characters were then compared with some literature, such as books and relevant scientific articles.[44]

Extraction and Cultivation

The isolated endophytic fungi were cultivated on Potato Dextrose Broth (PDB) media in 15 culture bottles (each of 350 ml). Cultures were incubated for 30 days at room temperature. After incubation, the biomass was separated from the media by using filter paper. Then, ethyl acetate solvent was added to the media at a ratio of 1:1 and extraction was carried out. The solvent was evaporated by using a rotary evaporator to obtain a concentrated extract.[45]

Antioxidant activity test

Endophytic fungi extracts were made in various concentrations (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL, and 15.625 μg/mL) in methanol. Furthermore, DPPH (2.2-diphenyl-1-picrylhydrazyl) was used to carry out the antioxidant activity test. As many as 0.2 ml of the extract concentration was homogenized with 3.8 ml of DPPH solution 0.5 mM and incubated in the dark for 30 minutes. Absorbance was measured using a UV-VIS spectrophotometer with a wavelength of 516 nm. Measurement of antioxidant activity was obtained from the percentage of DPPH inhibition and IC_{50} value.[20,46,47]

Antibacterial Activity Test

The disc diffusion method was used for antibacterial activity test. The bacteria used were *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus subtilis* while the medium used was Muller Hinton Agar (MHA). As much as 400 μg/disc of endophytic fungi extract was dripped onto the disc paper. Tetracycline was used as a positive control with a concentration of 30 μg/disc. The paper discs that had been treated were placed on the media that had been inoculated with the test bacteria and then incubated for 1 x 24 hours at 37°C. After incubation, the inhibition zone was observed and measured using a caliper. Determination of the criteria for antibacterial activity using the following formula:[48]

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

A: Inhibition zone of sample
B: Inhibition zone of positive control

Identification of Endophytic Fungi Molecularly

The best antioxidant and antibacterial of endophytic fungi extract were identified molecularly based on the ITS rDNA area. The amplification used ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') as primers. The sample sequences were then BLAST-ed on the http://blast.ncbi.nlm.nih.gov/Blast.cgi. Sample sequences and databases were aligned by CLUSTAL-W method in the MEGA11 program. Phylogenetic tree reconstruction using neighbor-joining with a bootstrap value of 1000.[49]

Compound Isolation and Identification

The selected endophytic fungi extracts were prepared and pre-absorbed, then evenly distributed into the chromatography column and eluted using an eluent with increasing polarity. Eluate was collected every 10 ml. Each column fraction was evaporated and then separated and purified by chromatographic techniques to obtain a pure compound. The chemical structures were identified by GC-MS and spectroscopy methods including ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC.

Result and Discussions

Characteristics of endophytic fungi isolated from Sungkai leaves

The endophytic fungi colonies found showed different characteristics, both macroscopic and microscopic (Figure 1). This study re-isolated endophytic fungi using a different method from previous studies,[20] namely by bruising plant organs. A total of 16 endophytic fungi isolates were isolated from the Sungkai leaves, and four isolates were identified that differed from those previously reported.[20] Because this bruising technique caused more damage to the surface area of plant cells, endophytic fungi that were not previously identified appeared in this study. It indicated that the isolation technique of endophytic fungi determined the variety and number of endophytic fungi. The four isolates had varying colony colors, such as white, black, and yellow. The results of macroscopic and microscopic observations can be seen in Table 1 and Table 2.

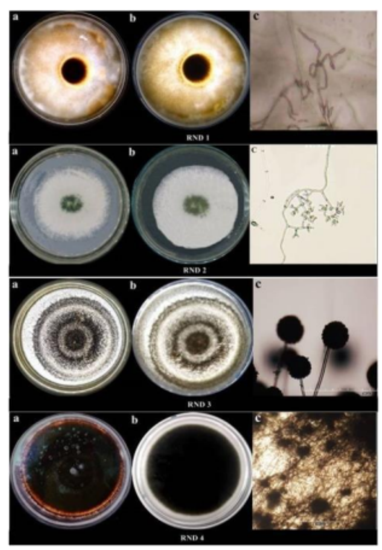

Figure 1: Macroscopic (a: front view; b: reverse view) and microscopic (c) characteristics

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Table 1: Colony characteristic of endophytic fungi isolated from Sungkai leaves

Code	Surface Colony	Reverse Colony	Structure	Elevation	Pattern	Exudate Drops	Radial line	Concentric circle
RND1	Grey with dark brown	Grey with dark brown	Cottony	rugose	zonate	-	√	-
RND2	White	White	Cottony	umbonate	radiate	-	√	-
RND3	Black with white	Black with White to cream	Powdery	Umbonate	radiate	-	-	√
RND4	Black	Black	Cottony	umbonate	radiate	-	-	-

Table 2: Microscopic characteristics of endophytic fungi isolated from Sungkai leaves

Isolate	Spore	Shape	Hyphae	Characteristic	Species of Identification
RND1	Hyaline	Subglobose	Septate	Conidiophores (phialides) hyaline, erect, gradually tapering from base towars apex.	*Paecilomyces variabilis*
RND2	Sporangia	Globose	Septate	Conidiophores hyaline, phialides short and thick, globose	*Trichoderma harzianum*
RND3	Conidia	Subglobose	Septate	Conidiophores long smooth, may be brownish near top. Phialides radiate around antire vesicle and are biseriate, with the metulae twice as long as the phalides.	*Aspergilus niger*
RND4	Conidia	Subglobose	Septate	Hyphae are septate, with conidiogenous cells.	*Phaeotrichosphaeria sivanesan*

Four species of endophytic fungi were found on Sungkai leaves with characteristics described in Table 1 and Table 2. These characterizations provided information for identifying endophytic fungi species. Based on these characteristics, the species of endophytic fungi found on Sungkai leaves were *Paecilomyces variabilis* (RND1), *Trichoderma harzianum* (RND2), *Aspergillus niger* (RND3), and *Phaeotrichosphaeria sivanesan* (RND4).

Bioactivity of Endophytic Fungi Extract

The ethyl acetate extract of endophytic fungi isolated from Sungkai leaves has potential as an antioxidant and antibacterial (Table 3). The four endophytic fungi extracts revealed antibacterial activity against the four tested bacteria and antioxidant activity. RND3 isolate extract showed very strong antioxidant activity and strong antibacterial activity against all four bacterial tests.

Table 3 describes the methanol extract of the host plant which has strong antioxidant activity and inhibits the growth of the four test bacteria. Host plant extracts showed bioactivity equivalent to their endophytic fungi extracts. However, based on its value, the methanol extract of Sungkai leaves had a lower antioxidant and antibacterial activity value compared to the RND3. Extract of RND3 revealed the best bioactivity compared to extracts of other endophytic fungi and their host plants. Therefore, molecular identification of RND3 isolates was carried out.

Molecular Identification of Endophytic Fungi

RND3 was the selected endophytic fungi for molecular identification due to its potential. This isolate can be used as a source of raw materials for medicines. The results of molecular identification of RND3 isolates can be seen in Figure 2 with the following sequences:

TTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGG
GTCCTTTGGGCCCAACCTCCCATCCGTGTCTATTGTACCCTG
TTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGC
GCCTCTGCCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACA
CGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATG
CAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGC
ATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAAT
TGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTG
CGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA
TTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCC
CCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACC
GCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTG
TAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTCTTT
CCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTT
AAGCATATCAATAAGCGGAGGA. Based on the identification, RND3 was *Aspergillus niger*.

Aspergillus niger is an opportunistic fungal pathogen that can be found under various conditions. The spores can easily spread through the air (aerosol) and have the potential to be inhaled by humans, through which they can enter the respiratory tract and cause the development of allergies.[50-52] However, despite its pathogenic nature, research has revealed that *A. niger*'s ability to spread quickly is associated with plants, and there is no information regarding its host specificity.[57–60] Fungi isolated from medicinal plants are known to have good bioactivity. This indicates that the *A. niger* found in Sungkai leaves should be capable of producing secondary metabolites that are also present in its host plant. Several studies have reported that the *A. niger* isolated from medicinal plants acts as an antimicrobial and antioxidant because the compounds it contains have structural similarities with their host plants. The hydroxyl and carbonyl groups contained in the compound cause antioxidant and antibacterial activity.[61–63]

The ethyl acetate extract of *A. niger* showed very strong antioxidant activity with an IC_{50} value close to ascorbic acid ($IC_{50} < 20$ µg/mL). Its antibacterial activity is in the strong category against the four test bacteria. *A. niger*, as endophytic fungi, is very promising to be used as raw material for new drugs, considering the limited raw materials and the current antibiotic resistance. This biological activity is related to that found in the compounds contained in the *A. niger* extract. This has a large number of cryptic biosynthetic gene clusters (BGCs) so that it is able to produce various biomolecules as secondary metabolites with a broad spectrum. The secondary metabolites contained therein include pyranone, alkaloids (purolles and pyridones), amides, cyclopeptides, polyketide (asperyellone), and sterols.[50,64,65] The content, especially the alkaloid group, is known to have the ability as an antioxidant and antibacterial.[66–69] The solvent used in this research is ethyl acetate which is a semipolar solvent. The ability of ethyl acetate to bind polar and nonpolar compounds so that the secondary metabolites contained in the extract are more varied.

Isolation and Identification of Secondary Metabolites

Aspergillus niger (RND3) was cultivated in 4.5 L of PDB medium (in 15 culture bottles) for 4 weeks, then the liquid culture was extracted by partitioning with ethyl acetate solvent. The evaporation result obtained

3.2 g of ethyl acetate extract. Ethyl acetate extract showed the presence of two dominant purple stains. Thus, the ethyl acetate extract is continued to the separation stage. Ethyl acetate extract (3 g) was preabsorbed and column chromatographed by gravity using n-hexane, ethyl acetate, and methanol eluent in a gradient manner and collected in 48 vials containing 10 mL each. After TLC, they were grouped into five column fractions (F1-F5). The F3 fraction showed a potential stain, so it was column chromatographed using n-hexane-ethyl acetate (5:5) eluent until 11 vials were obtained and in TLC. The results can be grouped into two column fractions, namely F3.1-F3.2. Fraction F3.2 with a potential stain was rinsed with n-hexane-ethyl acetate (5:5) until compound 1 was obtained in the form of white crystals of 45 mg. Spectroscopic analysis of compound 1 can be seen in Figure 3.

The NMR spectrum of compound 1 (Figure 3) shows the presence of 23 proton signals. There are three proton signals in the aromatic region δ_H 7.16 (2H, d, J= 7 Hz); 7.19 (1H, d, J= 7 Hz); and 7.27 ppm (2H, t, J= 7 Hz) for a total of five aromatic protons. There were two signals, each of which integrated to two protons, doublet splitting with a coupling constant of 7.0 (ortho). These three signals indicated that compound 1 was a mono-substituted aromatic compound. There were eight signals in the region of vinylic protons and oxygenated protons, namely at δ_H 3.72 – 5.94 ppm. Furthermore, there were four methyl signals, namely at δ_H 2.27 (3H, s); 1.46 (3H, s); 1.11 (3H, d, J= 6.5 Hz); and 0.52 ppm (3H, d, J= 7 Hz). In addition, there were four signals for two sp^3 methylene groups and four sp^3 methine proton signals.

The ^{13}C-NMR and HMQC spectra of compound 1 (Figure 3 B and C) reveal the presence of 27 carbon signals. The HMQC spectrum (Figure 3 C) shows 23 ^{1}H-^{13}C correlations through one bond. There were 13 sp^2 carbon signals that appear at $\delta_C > 100$ ppm consisting of three carbon signals that were in low fields, namely at δ_C 210.3 ppm as ketone carbonyl carbon; 175.3 as the carbonyl carbon of carboxylic acids; and 170.4 ppm as the ester carbonyl carbon. Furthermore, there were four aromatic carbon signals, two of which are equivalent with high intensity. This proved that compound 1 was a monosubstituted aromatic. The other six sp^2 carbon signals appeared as a methylene carbon, three methine carbons, and a quaternary sp^2 carbon. In addition there were two signal oxygenated methine sp^3 carbons, four signal methine sp^3 carbons, and two sp^3 quaternary carbons.

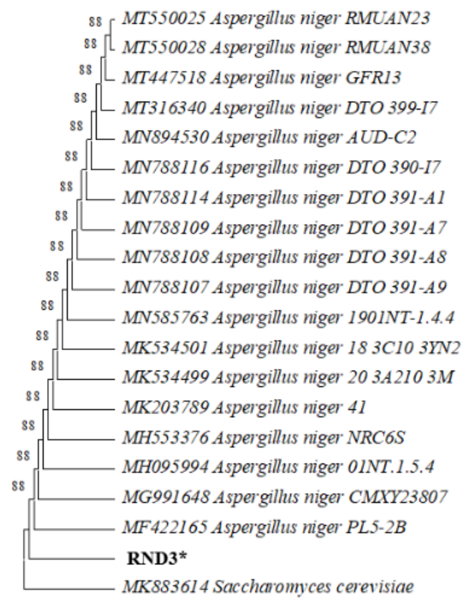

Figure 2: Phylogenetic tree of RND3 isolates (signed*) constructed by using the Neighbor-Joining method (bootstrap value = 1000).

Table 3: Percentage of antibacterial activity and IC_{50} of endophytic fungi extract isolated from Sungkai leaves compared to positive controls

Code of Isolate	Species	Antibacterial Activity (%)				Antioxidant Activity IC_{50} (µg/mL)
		E. coli	*S. aureus*	*S. typhi*	*B. subtilis*	
Methanol Extract of Sungkai Leaves		76.8 ± 0.71***	80.2 ± 0.46***	74.9 ± 0.64***	79.7 ± 0.49***	19.36***
RND1	*Paecilomyces variabilis*	72.1±0.48***	77.1±0.94***	87.9±0.77***	72.1±0.48***	84.66 ***
RND2	*Trichoderma harzianum*	72.4±1.07***	60.0± 0.55**	72.9± 0.51***	87.8± 0.40***	23.81 ***
RND3	*Aspergilus niger*	81.1± 0.38***	88.0±0.90***	87±0.38***	78.1±0.44***	15.39 ****
RND4	*Phaeotrichosphaeria sivanesan*	78.4± 0.17***	67.3±0.11**	60.0± 0.55**	72.9± 0.51***	505.97 *
Positive Control		Tetracycline 100***	Tetracycline 100***	Tetracycline 100***	Tetracycline 100***	Ascorbic Acid 10.083****

Note: Antibacterial activity percentage: *** ≥ 70% (strong), **50-70% (moderate), and *< 50% (weak).
Antioxidant activity IC50 (µg/mL): ****very strong <20 µg/mL ***strong < 100 µg/mL; **moderat 100-500 µg/mL; * weak > 500 µg/mL

The HMBC spectrum (Figure 4) shows the correlation of 22 protons to the carbon atom via two or three bonds. NMR spectrum data of 1D and 2D of compound 1 were shown in Table 4. The HMBC correlation of compound 1 was shown in Figure 5.

This study found one compound produced by A. *niger* isolated from Sungkai leaves (Figure 6). This compound showed weak antioxidant activity (IC_{50} > 500 µg/mL) and strong antibacterial activity against the four tested bacteria (MIC ≤ 64 µg/mL) (Table 5). This compound has lactone, carbonyl, and hydroxyl groups (Figure 6). The lactone group is very important for antibacterial activity. Lactones that have side chains can increase the compound's ability to bind to the bacterial cell wall, causing damage to the wall.[70–72] Likewise, the mechanism of the carbonyl group is capable of acting as an antibacterial. The compounds in this study showed the presence of hydroxyl groups, which play an important role in antioxidant activity.[73–75] The compounds in this study showed the presence of hydroxyl groups. This hydroxyl group plays an important role in antioxidant activity. However, the small number of hydroxyl groups may have caused this compound to display weak antioxidant activity. Several studies have shown that hydroxyl groups at specific positions on the aromatic ring can increase antioxidant activity.[76–80] Thus, the position of the hydroxyl group greatly influences the ability of a compound to scavenge free radicals. Research has explained that the removal of hydroxyl groups decreases the ability of compounds to scavenge free radicals.[81]

Table 4: The NMR data of compound 1, recorded at [1]H-500 MHz; [13]C-125 MHz in CD_3OD

No	δ_C ppm	Type of C	δ_H ppm (ΣH. Multiplicity (Hz))	HMBC	COSY
1	53.9	C			
2	76.8	CH	5.39 (1H, t, J= 2 Hz)	127.5; 132.1; 46.4; 53.6	5.22; 5.94
3	53.6	CH	3.25 (1H, m)	32.1	
4	32.1	CH	2.58 (1H, m)	149.5; 12.1k; 53.6	2.14
5	149.5	C			
6	71.0	CH	3.72 (1H, d, J= 10 Hz)	112.4; 130.4; 149.5	2.83
7	46.4	CH	2.83 (1H, d, J= 10 Hz)	175.3; 133.0; 71.0	3.72
8	130.4	CH	5.52 (1H, dd, J= 8.5; 15.50 Hz)	38.0	5.25
9	133.0	CH	5.25 (1H, m)		5.52
10	38.0	CH_2	2.34 (1H, q, J= 11 Hz)	130.4; 133.0; 42.0; 18.4;	2.79; 1.97
			1.97 (1H, t, J= 2 Hz)	42.0	2.34
11	42.0	CH	2.79 (1H, m)		1.11
12	210.3	C			
13	78.0	C			
15	170.4	C			
16	127.5	CH	5.22 (1H, dd, J= 2.5; 12.50 Hz)	78.0	5.94; 5.39
17	132.1	CH	5.94 (1H, dd, J= 2.5; 15.75 Hz)	127.5; 76.8	5.22; 5.39
18	19.3	CH_3	2.27 (3H, s)	170.4	
19	12.1	CH_3	0.52 (3H, d, J= 7 Hz)	149.5; 32.1	2.58
20	112.4	CH_2	5.14 (1H, s wide)	71.0; 32.1	4.95
			4.95 (1H, s wide)	71.0; 32.1	5.14
21	175.3	C			
22	18.4	CH_3	1.11 (3H, d, J= 6.5 Hz)	210.3; 38.0; 42.0	2.79
23	23.2	CH_3	1.46 (3H, s)	210.3; 53.6	
24	43.5	CH_2	2.85 (1H, dd, J= 8.5; 5.5 Hz)	129.6; 137.0; 53.6	2.67
			2.67 (1H, dd, J= 8.5; 13.0 Hz)	129.6; 137.0; 53.6	2.85
1'	137.0	C			
2'	129.6	CH	7.16 (2H, d, J= 7 Hz)	126.5; 129.6; 43.5	
3'	128.3	CH	7.27 (2H, t, J= 7 Hz)	128.3; 137.0	
4'	126.5	CH	7.19 (1H, d, J= 7 Hz)	129.6	
5'	128.3	CH	7.27 (2H, t, J= 7 Hz)	128.3; 137.0	
6'	129.6	CH	7.16 (2H, d, J= 7 Hz)	126.5; 129.6; 43.5	

Table 5: MIC and IC_{50} values of compound 1 from *Aspergillus niger* compared to tetracycline and ascorbic acid as standards

Sample	MIC Values (µg/mL)				Antioxidant Activity IC_{50} (µg/mL)
	E. coli	***S. aureus***	***S. thypi***	***B. subtilis***	
Compound 1	32	32	64	64	> 500
Tetracycline[a]	4	4	4	4	
Ascorbic Acid[b]					10,1

Note: [a] positive control of antibacterial; [b] positive control of antioxidant

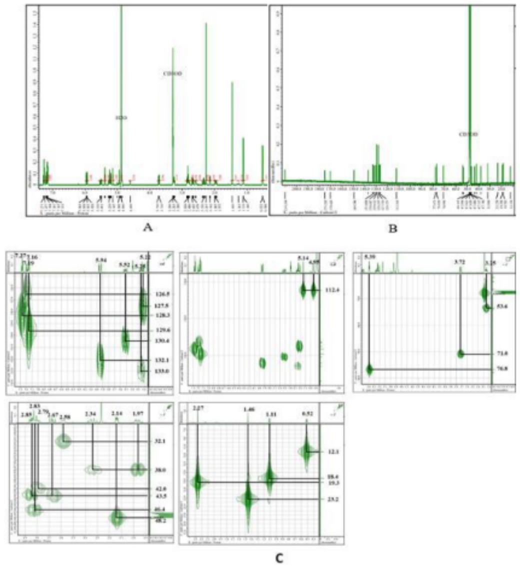

Figure 3: The ^{1}H-NMR (A), ^{13}C-NMR (B) and HMQC (C) spectra of compound 1 (^{1}H-500 MHz; ^{13}C-125 MHz in CD_3OD)

Conclusion

Four endophytic fungi were found in this study to complete the previously reported endophytic fungi from Sungkai leaves. Extract of *Aspergillus niger* had antibacterial and antioxidant activity but its pure compound had weak antioxidant activity. In future studies, the isolation of antioxidant compound that have not been reported so far will be carried out.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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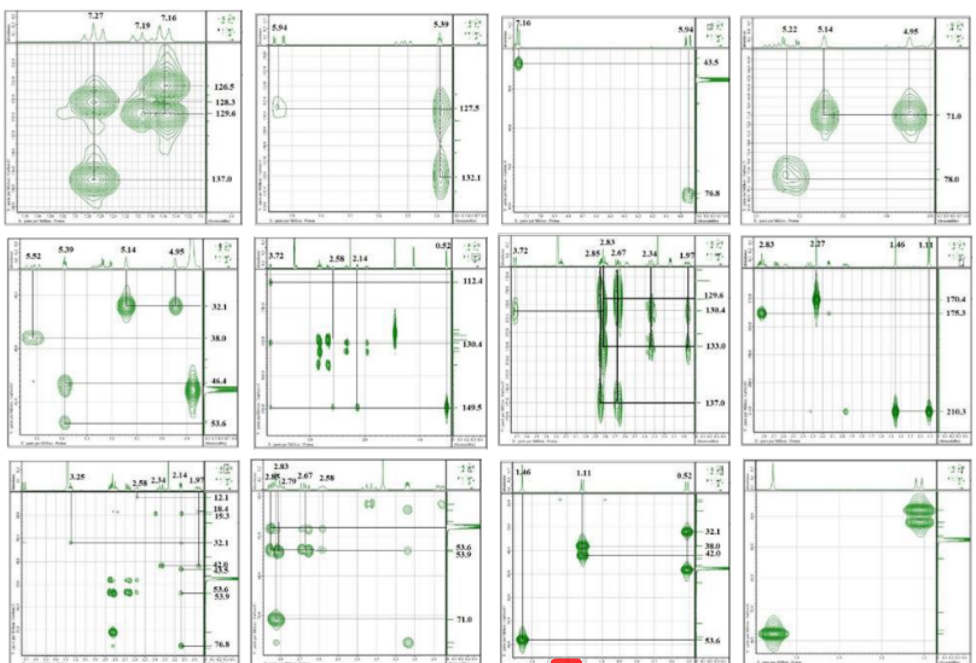

Figure 4: The HMBC spectra of compound 1

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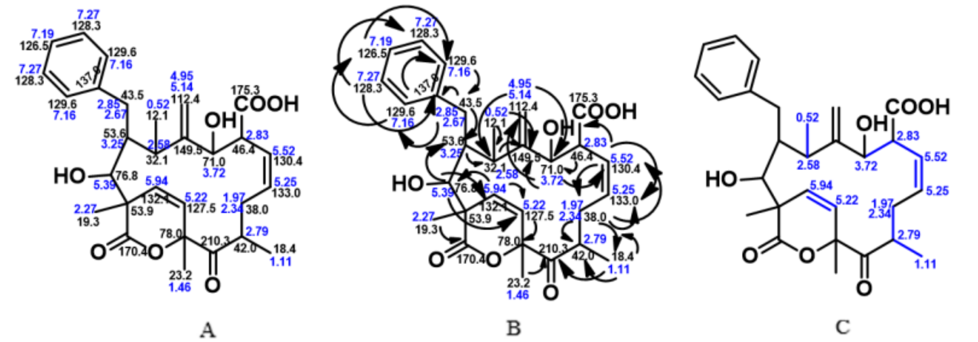

Figure 5: The placement of carbon and proton chemical shifts (A), HMBC correlation (B), and COSY correlation (C) of compound 1

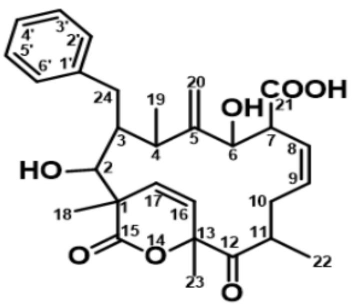

Figure 6: The structure of compound 1 as: 3-benzyl-2,6-dihydroxy-1,4,11,13-tetramethyl-5-methylene-12,15-dioxo-14-oxabicycloheptadeca-8,16-diene-7-carboxylic acid

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