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Research Paper

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

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Abstract
Peronema canescens, often known as sungkai, is widely used and can be found all around Indonesia. The public believes that the leaves may reduce fever and strengthen the immune system. However, the effectiveness of sungkai fruit has not been thoroughly investigated. In this research, we looked at endophytic fungus extracts from sungkai fruit's that have antioxidant and antibacterial properties. The study's results will serve as the foundation for further investigation into the development of potential natural chemicals with antioxidant and antibacterial properties. Morphologically, the endophytic fungi isolated from sungkai fruit were identified. The antioxidant and antibacterial properties of endophytic fungal extracts were studied using the DPPH technique and the paper disk diffusion method. By employing molecular identification and column chromatography to separate the active compounds, the most likely endophytic fungal isolates were found based on the results of the bioactivity tests. Using 1D NMR spectroscopic methods, the chemical's structure was determined, and the results were compared to NMR data for the same compound published in the literature. Fruit of sungkai had 8 strains of endophytic fungus (RBH1-RBH8). Strong antibacterial and very strong antioxidant activity were shown by the RBH5 isolate (IC_{50} < 20 μg/mL). *Pythium periplocum* was determined to be the RBH5 isolate based on molecular testing. Pure chemical compound extracted from RBH5 isolates shown highly potent and potent antibacterial and antioxidant effects. The chemical compound was identified by spectroscopy as 3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-γ-butyrolactone. The results of this study serve as the foundation for developing compounds as pharmaceutical raw materials via further research phases.

Keywords
Antibacterial, Antioxidant, Endophytic Fungi, Fruit of Sungkai, Secondary Metabolite

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1. INTRODUCTION

In Indonesia, using medicinal herbs has been prevalent for a very long time, not just in rural but also in metropolitan regions (Bouqoufi et al., 2023; Pradipta et al., 2023). The community is concerned about the spread of infectious illnesses like Covid-19 and thinks that medicinal herbs can stop it or possibly cure it. People like sungkai as a herb that can lessen symptoms or perhaps cure it (Onyeaghala et al., 2023; Elfita et al., 2014; Khanna et al., 2021; Latief et al., 2021b; Oktiansyah et al., 2023a; Villena-Tejada et al., 2021).

Sungkai (P. *canescens*) is frequently used by the community as a traditional medicine by preparing it into basic components because it is effective in treating fever, illness, cough, and malaria (Elfita et al., 2023; Latief et al., 2021a; Ocan et al., 2023; Oktiansyah et al., 2023b). The secondary metabolites found in this plant include sitosterol, alkaloids, saponins, tannins, terpenoids, diterpenoids, and flavonoids. The bioactivities of these secondary metabolites have been described as having antibacterial, antimalarial, antioxidant, antidiabetic, and uric acid-lowering properties (Oktiansyah et al., 2023c; Rahardhian et al., 2022). On the other hand, less research has been done on the sungkai fruit's secondary metabolite composition. The local population also uses the sungkai fruit as a traditional medicinal component. The boiled water of the fruit and leaves is used by the people to treat fever. The lengthy sungkai plant fertilization process and the fruit's limited resources make it difficult to study its secondary metabolites. Endophytic fungal biotechnology is needed to examine the secondary metabolites of the Sungkai fruit.

Fungal symbioses with plant tissues are known as endophytic fungi (Hashem et al., 2023; Lavado and Chiocchio, 2023; Poveda Arias et al., 2022; Priyashantha et al., 2023). Because of these interactions, endophytic fungi may replicate and even alter compounds from their host plants (Musa et al., 2023; Song et al., 2023). It is thought that The key findings to meet the demand for new medicinal substances are the secondary metabolites of these endophytic fungus. This creates a chance to beat the synthetic antioxidants and antibiotics already in use (Alam et al., 2021; García-Latorre et al., 2023; Gupta et al., 2023; Liu et al., 2023; Wen et al., 2022; Xu et al., 2021). According to studies, most of the endophytic fungus-produced compounds found in medicinal plants have a certain structure and are just as bioactive as their host bodies or perhaps more active (Caruso et al., 2022; Dos Reis et al., 2022; Shen et al., 2023; Srinivasa et al., 2022; Tan et al., 2022). study on endophytic fungus found in the sungkai plant's various sections has also been reported, such as leaves, stems and roots. A total of 12 isolates of endophytic fungi were obtained from sungkai leaves and it was found that the 3-(2,6-dihydroxyphenyl)-2-hydroxyacrylic acid compound isolated from *Penicillium oxalicum* showed strong antibacterial and antioxidant activity (Elfita et al., 2022). Then, 4 isolates of endophytic fungi were obtained from sungkai root bark, where *Penicillium janczewskii* showed strong antioxidant and antibacterial activity whose chemical compounds had not yet been reported (Oktiansyah et al., 2023a). In addition, 20 endophytic fungal isolates were isolated from sungkai stem bark, with *Curvularia intermedia* and *Colletotrichum cliviicola* as the fungi with the most potential antioxidant and antibacterial activity. Both fungi produce two compounds, namely 3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-γ-butyrolactone (1) and 5-hydroxy-4-(hydroxymethyl)-2H-pyran-2-one (2), where both compounds are active as antibacterials and antioxidants. Based on these research, endophytic fungi isolated from sungkai fruit are thought to have antioxidant and antibacterial activity. Therefore, the purpose of this research is to explore secondary metabolites with antioxidant and antibacterial activity that have been discovered from several types of endophytic fungus found in medicinal plants, in this case the fruit of the sungkai.

2. EXPERIMENTAL SECTION

2.1 Materials and Method

The materials to conduct this study were Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB) from Oxoid, alcohol 70%, NaOCl solution from Onemed, TLC Si Gel plates (Merck kieselgel 60 GF254, 0.25 mm, 20 x 20 cm, column chromatography using Merck Si Gel 60 (70–230 mesh). The organic solvents used are n-hexane, ethyl acetate, methanol, aquabidest, DPPH (D9132 Sigma-Aldrich). The antioxidant and antibacterial properties of endophytic fungal extracts were studied using the DPPH technique and the paper disc diffusion method.

2.2 Instrumentation and Characterization

Antioxidant activity test used spectrophotometer UV-Vis (Shimadzu UV-1900) to check the absorption of DPPH while antibacterial activity test used Laminar Air Flow (LVG-4AG-F8) to avoid the contamination. The characterization of chemical compounds using a NMR spectrum on JEOL JNM-ECZ500R/S1 500 MHz (1H); 125 MHz (13C).

2.3 Procedure

2.3.1 Processing of the Sample and Isolation of Endophytic Fungus

The collected sample's identity was assigned the prefix 302/UN 9.1.7/4/EP/2021 at the Plant Systematics Laboratory, University of Sriwijaya. Before isolating the endophytic fungus, the fresh fruit's surface was first sanitized by being submerged in water for around 5 minutes. The sample was then submerged in 70% alcohol for about three minutes, washed with sterilized distilled water for about one minute, then submerged in a solution of 3% NaOCl for about one minute. Before being injected onto a petri plate with PDA, the sample was first sliced aseptically into three 1 cm squares. The inoculants were incubated in room settings for 3 to 14 days. Transferring the colonies to a fresh medium in petridish and culturing them there for 48 hours will purify the endophytic fungus (Khalil et al., 2021; Talukdar et al., 2021; Widjajanti et al., 2022).

2.3.2 Morphological Characterization and Identification of Fungal Endophytes

Endophytic fungal were identified using phenotypic traits. With a 1000× magnification, microscopic properties were observed using the slide culture method. For identification purposes, the created macroscopic and microscopic phenotypic features were subsequently analyzed using a variety of sources (books and journals) (Noman et al., 2023; Thitla et al., 2023; Pitt and Hocking, 2009; Walsh et al., 2018; Watanabe, 2010).

2.3.3 Cultivation and Extraction

Six blocks of agar (6 mm in diameter) were used for cultivation, and 300 mL of potato dextrose broth medium were applied to hold the pure cultures of the endophytic fungi isolates that were produced. 15 glass bottles totaling 1 L were used to grow the isolate. After that, the cultures were cultured statically for 1 month at room temperature. This condition is very good for fungi to absorb nutrients optimally for their development. Using filter paper to remove the media from the fungal biomass, the culture medium was then reconstituted with ethyl acetate in a 1:1 ratio. The extracts were detached using a rotary evaporator after 10 days (Dos Reis et al., 2022; Wang et al., 2023).

2.3.4 Antioxidant Activity Test

The DPPH technique was employed to measure antioxidant activity, adding 0.5 mM DPPH solution volume to 0.2 mL of each extract concentration (Baliyan et al., 2022). A dark tube was used to incubate the blend solution for 30 minutes.

Ascorbic acid was used as a reference and In order to quantify the absorbance, a spectrophotometer was used at 517 nm. Calculations were made to assess antioxidant activity using the percentage of inhibition and IC_{50} value (Abbas et al., 2021).

$$\% \text{ Inhibition} = \frac{A_k - A_s}{A_s} \times 100 \quad (1)$$

A_k = Control
A_s = Samples

2.3.5 Antibacterial Activity Test

The paper disc diffusion technique was employed to assess the antibacterial activity. MHA (Muller Hinton Agar) is the medium that is utilized. *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus* were the test microbes utilized. To determine the Minimum Inhibitory Concentration (MIC), concentrations of 256, 128, 64, 32, 16, 8, and 4 μg/mL were dripped onto blank disc paper. This concentration was chosen according to the test parameter, namely MIC, which aims to see the minimum concentration inhibited bacterial growth. Strong antibacterial medication with a MIC value of less than 100 μg/mL was identified (Ding et al., 2019).

2.3.6 Fungal Endophytes' Molecular Identification

The endophytic fungal isolates with the highest potential for bioactivity were used for Molecular identification. The ITS DNA (rDNA) region was employed for identification. The amplification process used the primers ITS1 and ITS4. BLAST (`http://blast.ncbi.nlm.nih.gov/Blast.cgi`) includes the sequences. Additionally, The CLUSTAL W method of the MEGA11 software was used to align the sequences, and a phylogenetic tree with a bootstrap value of 1000 was created using the Neighbour-joining tree method (Tamura et al., 2021).

2.3.7 Compound Isolation and Identification

Preabsorption was used to obtain the strongest extract, which was then applied to the chromatographic column. After that, the extract was eluted with a more polar eluent. A TLC test was run to separate the eluate into column fractions after it had been collected into a 10 mL vial. Using chromatographic procedures, column fractions are evaporated, separated, and purified to produce pure substances. The 1D NMR spectroscopy approach, which includes ^{1}H-NMR and ^{13}C-NMR were utilized to identify the chemical structure.

3. RESULTS AND DISCUSSION

3.1 Morphological Characterization and Identification of Fungal Endophytes

The discovered endophytic fungal colonies displayed several macroscopic and microscopic traits (Figure 1). The eight endophytic fungus isolates that were discovered had colonies of different hues, including white, yellow, and gray. Tables 1 and 2 show the findings of macroscopic and microscopic observations.

Eight different endophytic fungi species have been identified on sungkai fruit, and Tables 1 and 2 summarize the colony morphological traits of each species. These traits can be used to distinguish between different endophytic fungus species. These traits allow us to identify the type of endophytic fungus that lives on sungkai fruit were *Achaetomium sp.* (RBH1), *Rhizoctonia sp.* (RBH2), *Mortierella nana* (RBH3), *Codinaea parva* (RBH4), *Pythium periplocum* (RBH5), *Pyrenochaeta terrestris* (RBH6), *Colletotrichum gleosporioides* (RBH7), *Mortierella sp.* (RBH8).

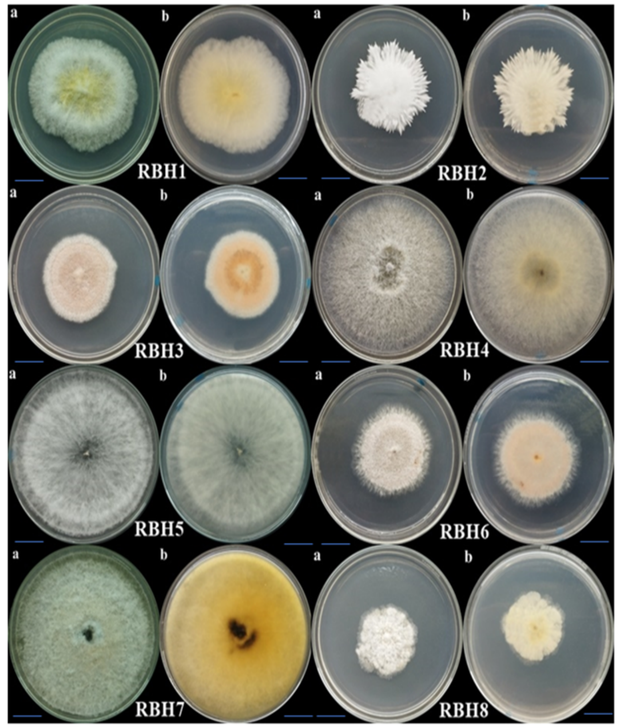

Figure 1. Macroscopic Characteristics of Endophytic Fungus Discovered in Sungkai Fruit (a: Front View; b: Reverse View. Scale Bar: 2 cm)

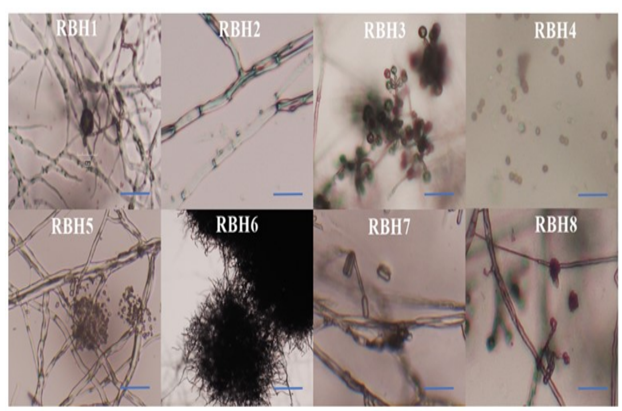

Figure 2. Microscopic Characteristics of Endophytic Fungus Discovered in Sungkai Fruit (Scale Bar: 200 μm)

Table 1. A Peculiar Colony Structure is Present in Endophytic Fungus Isolated from Sungkai Fruit

Code	Surface Colony	Reverse Colony	Structure	Elevation	Pattern	Exudate Drops	Radial Line	Concentric Circle
RBH1	White Yellowish	White Yellowish	Cottony	Umbonate	Radiate	-	✓	-
RBH2	White	White	Cottony	Umbonate	Radiate	-	✓	-
RBH3	Grey	White Greyish	Cottony	Rugose	Zonate	-	✓	✓
RBH4	White	White	Cottony	Umbonate	Radiate	-	✓	-
RBH5	White	White	Cottony	Umbonate	Zonate	-	-	✓
RBH6	White	White	Cottony	Rugose	Zonate	-	✓	✓
RBH7	White	White Yellowish	Cottony	Umbonate	Radiate	-	✓	-
RBH8	White	White	Cottony	Rugose	Zonate	-	-	-

Table 2. Microscopical Features of Endophytic Fungus Discovered in Sungkai Fruit

Isolate	Spore	Shape	Hyphae	Characteristic	Species of Identification
RBH1	Sporangia	Globose	Septate	Conidiophores hyaline, phialides short and thick, globose	*Achaetomium sp.*
RBH2	Not formed	Not formed	Septate	Conidia not formed, septated closely near the main hyphae	*Rhizoctonis sp.*
RBH3	Sporangia	Globose	Septate	Sporangiophores hyaline, forming vesicles	*Mortierella nana*
RBH4	Conidia	Phialides	Septate	Conidiospores erect, simple or rarely branched once	*Codinaea parva*
RBH5	Sporangia	Cylindrical	Septate	Sporangia lobate, terminar intercalary, zoospores rarely or discharged	*Pythium periplocum*
RBH6	Conidia	Globose	Septate	Shaped like a thorn,	*Pyrenochaeta terresris*
RBH7	Conidia	Subglobose	Septate	Conidiophores hyaline, branched, phialides short and thick	*Collectotrichum gloeosporioides*
RBH8	Sporangia	Globose	Septate	Conidiophores hyaline,	*Mortierella sp.*

3.2 Antioxidant and Antibacterial Activity of Endophytic Fungi Extract

Endophytic fungus isolated from sungkai fruit shown antioxidant and antibacterial properties in an ethyl acetate extract (Table 3). Several extracts of endophytic fungal showed antioxidant effects as well as antibacterial activity against the four test microorganisms. Against the four studied microorganisms, RBH5 isolate extract shown extremely significant bioactivity.

The methanol extract of the host plant, sungkai fruit, is described in Table 3 has potent antibacterial properties and inhibiting the growth of the four test microorganisms. The bioactivity of host plant extracts was comparable to that of their endophytic fungus extracts. In contrast to the RBH5 endophytic fungus extract, the Sungkai fruit's methanol extract had very strong antioxidant and antibacterial activities. RBH5 and its host plants had very strong bioactivity. The antioxidant

Table 3. The Endophytic Fungi Extract from Sungkai Fruit Compared to Ascorbic Acid and Tetracycline as Positive Controls for Antioxidant and Antibacterial Properties

Sample	Extract	Weight (gram)	% Antibacterial Activity *E. coli*	*S. aureus*	*S. thypi*	*B. subtilis*	Antioxidant Activity IC_{50} ± SD (µg/mL)
Host Plant	Methanol of Sungkai Fruit	2.1	77.56 ± 0.43 ***	81.45 ± 0.51 ***	79.54 ± 0.68 ***	80.6 ± 0.34 ***	15.36 ****
Endophytic Fungi	RBH1	1.2	68.1 ± 0.48 **	77.1 ± 0.94 ***	77.9 ± 0,77 ***	80.9 ± 0.22 ***	233.49 *
	RBH2	1.2	74.8 ± 0.18 ***	85.5 ± 1.62 ***	75.2 ± 0.35 ***	89.4 ± 1.68 ***	29.44 ***
	RBH3	1.4	61.3 ± 1.11 **	65.3 ± 0.39 **	75.2 ± 0.05 ***	74.7 ± 0.31 ***	32.63 ***
	RBH4	1.2	68.1 ± 0.72 **	78.6 ± 0.84 ***	73.0 ± 0.75 ***	62.6 ± 0.11 **	78.59 ***
	RBH5	2.0	90.2 ± 0.47 ***	89.4 ±0.07 ***	90.5 ± 0.40 ***	88.7 ± 0.83 ***	18.14 ****
	RBH6	2.1	85.5 ± 0.69 ***	79.9 ± 0.09 ***	88.3 ± 0.68 ***	79.2 ± 0.46 ***	115.62 ***
	RBH7	1.2	61.3 ± 0.36 **	73.0 ± 0.86 ***	72.5 ± 0.84 ***	61.3 ± 0.81 ***	25.41 ***
	RBH8	1.8	73.8 ± 0.24 ***	78.8 ± 0.78 ***	73.7 ± 0.92 ***	78.7 ± 0.68 ***	21.15***
Positive Control			Tetracycline 100 ***	Tetracycline 100 ***	Tetracycline 100 ***	Tetracycline 100 ***	Ascorbis Acid 10.1 ****

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

activity of host plants, based on IC_{50} values, was indeed better than RBH5. It was suggested that the compound composition in the host plant is slightly greater than in the endophytic fungi. However, The IC_{50} value shows that both substances have extremely potent antioxidant. Comparing RBH5 isolation extract to other endophytic mushroom extracts, it had the excellent bioactivity so RBH5 can be identified molecularly.

3.3 Molecular Identification of Endophytic Extract

The endophytic fungal isolate chosen for molecular identification was RBH5. RBH5 isolate had the highest potential outcomes, according to the bioactivity test findings. Because of its potential, this isolation can be utilized to produce pharmaceutical raw materials. Figure 2 displays the outcomes of the molecular identification of RBH5 isolates with the subsequent sequencing:

CGTCTTCTTGTAAGATTTGAGGCTGAACGAAGGT GAGTCTGCGTCTATTTTGGATGCGGATTTGCTGAT GTTATTTTAAACACCTATTACTTAATACTGAACTATA CTCCGAATACGAAAGTTTTTGGTTTTAACAATTAAC AACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGA TGAAGATCGCTGCGAACTGCGATACGTAATGCGAAT TGCAGAATTCAGTGAGTCATCGAAATTTTGAACGCA TATTGCACTTTCGGGTTATGCCTGGAAGTATTCCTG TATCAGTGTCCGTACATCAAACTTGTCTTTCTTTTT TTGTGTAGTCAAAATTAGAGATGGCAGAATGTGAGG TGTCTCGCGTTGTCTTTTTTTAAAAGATGGTTCGAG TCCCTTTAAATGTACGTTGATTCTTTCTTGTGTCTG CGAATTGCGACGCTATGCTCTTTGTGATCGGTTTAG ATTGCTTTGCGCTGGTGGGCGACTTCGGTTAGGAC ATATGGAAGCAACCTCAATTGGCGGTCTGTTCGGC TTTGCCTGACGTTAAGCTAAGCGAGTGTGGTTTTCT GTCTTTTCCTTGAGGTGTACCTATTGTGTGTGAGGT TGATGTAGACTATATGGTTGCTTGGTTGTGTGGTTT AGCGTTTTCAGACGCCTGCTTCGGTAGGTAAAGGAG ACAACACCAATTTGGGACTGAGAGTTA.

P. periplocum was first described as a plant pathogen of *C. vulgaris* that causes flower end rot disease. Apart from being found as a parasite on plants, the fungus *P. periplocum* is found in soil as its main habitat. However, several studies have revealed the mycoparasitic abilities of this fungus. Our research found that the fungus *P. periplocum* lives as an endophytic fungus on sungkai fruit. The bioactivity of endophytic fungus isolated from medicinal plants is quite high, as is well known (Brazkova et al., 2022; Chugh et al., 2022; Rehman et al., 2022; Vaou et al., 2022). This shows that *P. periplocum*, a fungus found in sungkai fruit, may generate secondary metabolites, which are thought to be present in the host plant as well.

3.4 Compound Isolation and Identification

Silica gel (1:1) was used to preabsorb the ethyl acetate extract RBH5 (2 g), and silica gel stationary phase was used to separate the mixture in column chromatography and eluted gradient with n-hexane:ethyl acetate (10:0 → 0:10) eluent and continued with ethyl acetate:methanol (10:0 → 0:10). Based on the

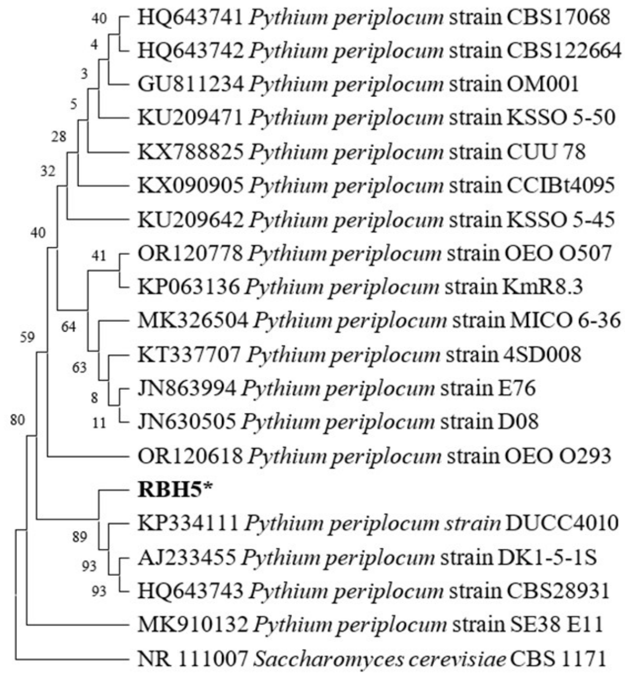

Figure 3. Neighbour-Joining was used to Create a Phylogenetic Tree for RBH5* with a Bootstrap Value of 1000

stain pattern on TLC, five subfractions were obtained (F1-F5). The F4 subfraction was then separated by column rechromatography with n-hexane:EtOAc (5:5 → 0:10) effluent until three subfractions were acquired (F4.1-F4.3). To create compound 1, subfraction F4.2 was washed with n-hexane:ethyl acetate (3:7) (55.6 mg).

The NMR spectrum of compound 1 (Figure 4) showed the presence of seven proton signals consisting of two aromatic signals (δ_H 7.00-8.50 ppm) which each had two proton integration and dublet fission (capling constant J = 9.0 Hz). Next there is a proton signal methine sp^3, and four proton signals bound to the oxygenated carbon atom (at δ_H 3.50-6.30 ppm).

The ^{13}C-NMR spectrum of compound 1 (Figure 5) showed the presence of nine carbon signals. There are five carbon signals at δ_C > 100 ppm as sp^2 carbon and four carbon signals at δ_C 55-75 ppm as sp^3 carbon. In the sp^2 carbon region there are two signals those are in the low field, namely at δ_C 165.2 as carbonyl ester carbon and at δ_C 150.3 ppm as oxyaryl carbon. Compound 1's ^{1}H-NMR and ^{13}C-NMR spectra were contrasted to identical NMR spectra from the literature, as indicated in Table 4.

Based on data analysis of ^{1}H-NMR and ^{13}C-NMR spectra, as well as comparison with the NMR spectrum of compounds which from the literature, compound 1 is an aromatic compound that has a parahydroxyl substituted benzene ring with 3-hydroxy-4-(hydroxymethyl)-γ-butyrolactone substituent. Consequently, it is suggested that the compound's chemical structure is 3-hydroxy-4(hydroxy(4-hydroxyphenyl)methyl)-

Table 4. The Compound 1 NMR Data (^{1}H-500 MHz; ^{13}C-125 MHz in CD3OD) Compared to 1* (^{1}H-500 MHz; ^{13}C-125 MHz in CDCl3)

No. C	σ_C ppm 1	σ_H ppm (Σ_H. Multiplicity, Hz) 1	σ_C ppm 1*	σ_H ppm (Σ_H. Multiplicity, Hz) 1*
1	150.3		150.3	
2	127.0	7.64 (1H, d, J= 9.0 Hz)	127.0	7.65 (1H, d, J= 9)
3	122.8	8.19 (1H, d, J= 9.0 Hz)	122.8	8.18 (1H, d, J= 9)
4	147.2		147.2	
5	122.8	8.19 (1H, d, J= 9.0 Hz)	122.8	8.18 (1H, d, J= 9)
6	127.0	7.64 (1H, d, J= 9.0 Hz)	127.0	7.65 (1H, d, J= 9)
2'	165.2		165.5	
3'	66.0	6.23 (1H, s)	66.0	6.24 (1H, s)
4'	57.2	4.13 (1H, m)	57.2	4.15 (1H, m)
5'	60.9	A. 3.80 (1H, m)	60.9	3.82 (1H, m)
		B. 3.62 (1H, m)		3.62 (1H, m)
6'	69.9	5.16 (1H, d, J= 2.5 Hz)	70.0	5.16 (1H, d, J= 2.5)

* (Elfita et al., 2023)

Table 5. The MIC and IC_{50} Values of the Ethyl Acetate Extract and Pure Components from the Endophytic Fungus *P. periplocum* were Calculated using the Standards Tetracycline and Ascorbic Acid

Samples	MIC Values (μg/mL) *E. coli*	*S. aureus*	*S. thypi*	*B. subtilis*	Antioxidant Activity IC_{50} (μg/mL)
EtOAc extract	64	64	32	32	18.14****
Compound	32	64	32	64	44.13***
Tetracycline[a]	4	4	4	4	
Ascorbic Acid[b]					10.08****

Note: [a]Antibacterial standard; antioxidant activity IC_{50} (μg/mL): ****very strong < 20 μg/mL ***strong < 100 μg/mL; **moderate 100-500 μg/mL; * weak > 500 μg/mL

γbutyrolactone as shown in Figure 6. The endophytic fungus *Curvularia intermedia*, which was isolated from Sungkai leaves, also contained the same substance (Elfita et al., 2023).

Table 5 displays the antioxidant and antibacterial activities of the pure component and EtOAc extract of *P. periplocum* in comparison to ascorbic acid and tetracycline as standards.

Table 5 displays the EtOAC extract of the endophytic fungi *P. periplocum* and its component were tested for their antioxidant and antibacterial characteristics. The findings demonstrated that the substances demonstrated strong antioxidant action (IC_{50} < 100 μg/mL) as well as strong antibacterial activity contrary to microorganisms (MIC ≤ 64 μg/mL). These findings suggested that the chemicals discovered may be used to create novel medicinal products. Recently, new sources of medicines are urgently needed. This aims to reduce the risk of resistance to microorganisms and synthetic ingredients (Gupta et al., 2023; Silva et al., 2022).

Strong antibacterial activity was demonstrated by the *P. periplocum* ethyl acetate extract (MIC ≤ 64 μg/mL). It had extremely significant antioxidant activity (IC_{50} < 20 μg/mL). The *P. periplocum* crude extract's high bioactivity was due to the metabolites it contained. According to studies, phenolic secondary metabolites make up the majority of *P. periplocum's* secondary metabolites (Bělonožníková et al., 2020). Phenolic substances can stop bacteria from growing and lessen oxidative stress. This substance can prevent bacteria from generating proteins and DNA by inhibiting ribonucleic acid reductase activity and decreasing the permeability of bacterial cell walls on the surface. These properties are provided by the hydroxyl group and long saturated side chain (Adamczak et al., 2019; Burel et al., 2021). According to the references, the host plant's compounds are identical to those found in the extract of endophytic fungus. This suggests that because of their function in mutualistic relationships, endophytic fungus can duplicate the their host plants' secondary metabolites. Additionally, phenolics serve as antioxidants.

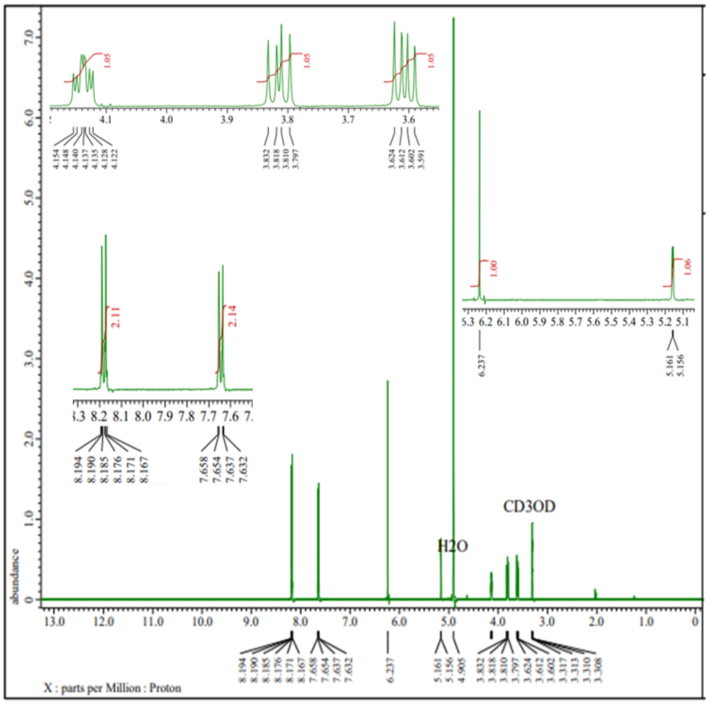

Figure 4. The Spectrum of Chemical 1's ^{1}H-NMR (1H-500 MHz in CD_3OD)

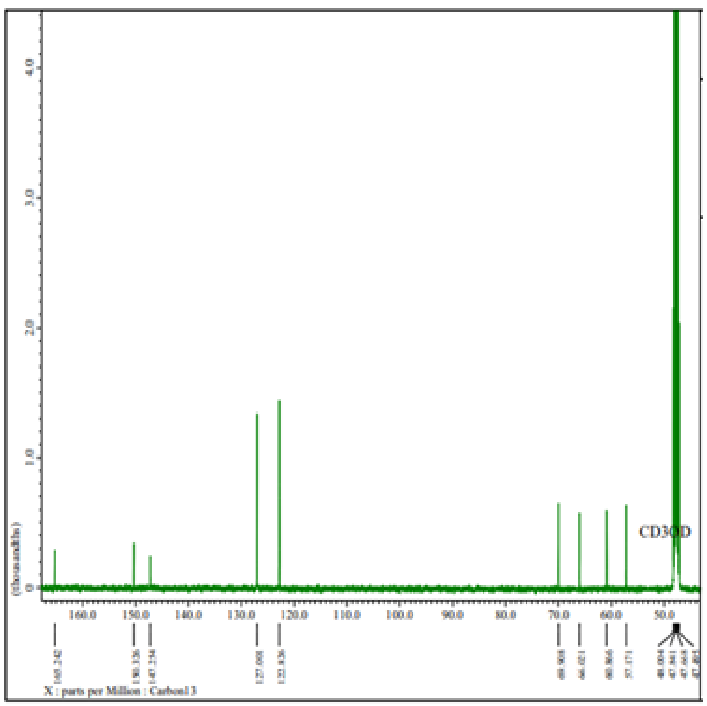

Figure 5. The ^{13}C-NMR Spectra of Compound 1 (^{13}C-125 MHz in CD_3OD)

The effectiveness of an antioxidant is closely related to the quantity of hydroxyl groups present, the reciprocal area of para in an aromatic ring, and the degree of esterification. According to studies, removing hydroxyl groups can decrease coplanarity, which can decrease a compound's capacity to stoke free radicals (Kubiak-Tomaszewska et al., 2022; Platzer et al., 2022). By substituting a methyl or glycosyl group for the hydroxyl group at its position (C3), quercetin's antioxidant

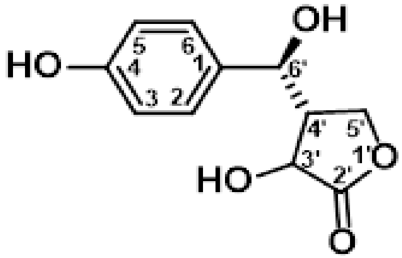

Figure 6. Chemical Structure of Compound 1

capabilities might be removed (Ferraz et al., 2020; Mucha et al., 2021). With the same number of hydroxyl groups connected to the aromatic ring, phenolic acids' antioxidant properties do not change noticeably (Spiegel et al., 2020). This suggests that a compound's antioxidant capabilities are strongly influenced by the placement of the hydroxyl group. 4-hydroxybenzoic acid, which is used in this study, contains a double band in the hydroxyl group, which makes it antioxidant activity.

4. CONCLUSION

Pythium periplocum, which was isolated from sungkai fruit, was shown to contain the bioactive chemical 3-hydroxy-4-(hydroxy (4-hydroxyphenyl)methyl)-butyrolactone. Additionally, endophytic fungi from the Sungkai leaves and stem bark also contain this substance. Strong antioxidant and antibacterial action is shown in this molecule. According to studies, this chemical compound can be modified in a number of ways to be used as a new medicinal substance.

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