https://doi.org/10.48048/tis.2025.9336

Bioactive Compound from Endophytic Fungus *Trichoderma* sp. Isolated from Cashew (*Anacardium occidentale*) Leaves

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Received: 15 November 2024, Revised: 3 December 2024, Accepted: 10 December 2024, Published: 28 February 2025

Abstract

Anacardium occidentale, commonly referred to as cashew, serves an important function in agriculture and healthcare sectors. The leaves of *A. occidentale* are valued as a vegetable and have applications in traditional medicinal practices. This study analyzed the antioxidant and antibacterial properties, along with bioactive compounds synthesized by endophytic fungi that exist in a symbiotic relationship within the leaf tissues of *A. occidentale*. The endophytic fungi were isolated from *A. occidentale* leaves using PDA (Potato Dextrose Agar) media and identified morphologically. Each pure isolate was cultured in Potato Dextrose Broth (PDB) medium for a duration of 4 weeks at ambient temperature under stable conditions. Following this incubation phase, the liquid culture was separated from the biomass and subjected to extraction with ethyl acetate, which was then evaporated to yield a concentrated extract. Each of these concentrated extracts was assessed for antioxidant properties using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and for antibacterial properties using the disk diffusion technique. Active extracts were further separated using chromatography techniques to isolate pure compounds, which were subsequently characterized by 1D and 2D NMR spectroscopy. This study identified 6 endophytic fungi (LM1 - LM6), comprising 3 *Trichoderma* sp. (LM1, LM2 and LM6), 1 *Aspergillus* sp. (LM3), 1 *Nigrospora* sp. (LM4) and 1 *Penicillium* sp. (LM5) based on morphological analysis. The antioxidant and antibacterial assays revealed that LM2 and LM4 demonstrated potent antioxidant effects and significant antibacterial efficacy against all bacterial strains tested. The LM2 isolate was selected for further bioactive compound isolation due to its higher extract yield. Chromatographic separation resulted in a pure yellow solid compound identified as a phenolic derivative, which showed weak antioxidant activity but demonstrated potent antibacterial effects against *Bacillus subtilis* and *Salmonella typhi*. Therefore, the endophytic fungus *Trichoderma* sp. (LM2) shows potential for development in the form of an extract or as a pure compound with structural modifications.

Keywords: Bioactive compound, Endophytic fungi, *Trichoderma* sp., *Anacardium occidentale*

Introduction

Anacardium occidentale, often identified as the cashew tree, is a tropical plant that holds significant importance in both agricultural and medicinal sectors. Native to northeastern Brazil, this species is extensively cultivated due to the high economic value of its seeds and fruit. Beyond its commercial significance, *A.*

occidentale has been widely acknowledged for its medicinal properties, which are attributed to its diverse phytochemical composition [1,2]. Different components of the plant, such as the roots, leaves, fruit and seeds, have long been used in traditional medicine to address a range of health issues, including inflammation, infections and gastrointestinal disorders. The broad spectrum of bioactive compounds present in *A. occidentale* has contributed to its reputation as a medicinal plant, fostering significant scientific interest in exploring its therapeutic potential [3-5].

The traditional use of *A. occidentale* is widespread, particularly in regions where the plant is native or cultivated. The roots are often used in herbal preparations to treat hypertension and malaria, while the leaves are applied topically for the healing of wounds and skin infections. The fruit, especially the cashew apple, is renowned for its anti-inflammatory and antimicrobial properties [6-8]. The seeds, commonly known as cashew nuts, are rich in essential fatty acids and antioxidants, which contribute to cardiovascular health [9,10].

Recent research has confirmed the potential of various parts of this plant as sources of bioactive compounds, revealing a diverse range of phytochemicals such as flavonoids, tannins and alkaloids. The leaves, for instance, contain high levels of phenolic acids known for their antioxidant and anti-inflammatory properties, while the fruit is an abundant source of vitamin C and other essential nutrients [5,6,8,11]. Root extracts have demonstrated significant antimicrobial activity, particularly against pathogenic bacteria. However, large-scale production of phytochemicals from *A. occidentale* is constrained by the availability of plant material, necessitating alternative strategies to meet the growing demand for natural therapeutics [9,12,13].

Biotechnology offers a promising solution to the challenges of sourcing bioactive compounds from *A. occidentale* by harnessing endophytic fungi, microorganisms that inhabit plant tissues without inducing any harm to the host. These fungi often produce secondary metabolites that are identical to or even more potent than those produced by their host plants, offering an alternative source for medicinal compounds [14,15]. By leveraging the metabolic potential of these fungi, researchers can develop scalable systems for producing valuable phytochemicals, thereby reducing dependence on plant resources and supporting biodiversity conservation [16].

Endophytic fungi offer several advantages, including the ability to be cultivated in controlled environments, thereby minimizing environmental impact. These fungi can produce a wide range of bioactive compounds, often with superior bioactivity and bioavailability compared to plant extracts [17,18]. Moreover, the use of these fungi overcomes the seasonal and geographical limitations associated with plant cultivation, ensuring a stable supply of bioactive compounds [19].

Previous studies have isolated 8 strains of endophytic fungi obtained from the leaves of *A. occidentale* (RDM1-RDM8), namely *Chrysosporium* sp., *Humicola tainanensis*, *Chaetomium* sp., *Phytophthora* sp. *Microsporum nanum*, *Rhizoctonia solani*, *Verticillium* sp. and *Neopestalotiopsis clavispora*. The ethyl acetate extracts derived from each of the 8 endophytic fungi exhibited notable antioxidant effects, with IC_{50} values between 13.80 and 84.66 µg/mL [20]. A more systematic investigation is needed to fully realize the potential of these endophytic fungi as sources for developing new therapeutic compounds. Therefore, this study further investigates the antioxidant and antibacterial activities, along with the chemical compounds synthesized by endophytic fungi residing symbiotically within the leaf tissues of *A. occidentale*. Continued research on the isolation of endophytic fungi, bioactivity assessment and cultivation of medicinal plants, particularly *A. occidentale*, could lead to the development of sustainable methods for producing new therapeutic agents. This approach aims to diversify the range of active pharmaceutical ingredients available to address the complexity of diseases prevalent in society today.

Materials and methods

Plant samples

The plant samples used in this study were leaves from the cashew tree (*A. occidentale*) obtained from Meranjat Ilir Village, Indralaya Selatan Subdistrict, Ogan Ilir Regency, South Sumatra Province. The plant samples were collected in a fresh state, free from any disease. The leaves selected were mature, dark green leaves, positioned as the third to fifth leaves from the

base. The samples were then placed in plastic bags and stored in a cool box containing blue ice during transportation from the collection site to the laboratory. Plant identification was conducted at the Generasi Biologi Indonesia Foundation (Genbinesia) with the certification number 08.115/Genbinesia/IX/2023.

Isolation of endophytic fungi

The leaves of *A. occidentale* were thoroughly washed under running water. Surface sterilization of the samples was performed in 3 stages. First, the samples were immersed in 70 % ethanol for approximately 2 min, followed by 3 % sodium hypochlorite (NaOCl) for 1 min. Finally, the samples were rinsed with distilled water for about 1 min and dried on sterile filter paper. The samples were cut with a sterile knife and placed on sterile PDA media, followed by incubation at 30 °C for a period of 2 to 7 days. Fungi with distinct morphologies were each inoculated onto PDA media to obtain pure isolates [21].

Identification of endophytic fungi

Pure isolates of endophytic fungi were identified using both macroscopic and microscopic analyses. Macroscopic characteristics were determined based on colony growth patterns, color, topography, texture, edge pattern, surface coloration and other relevant attributes. For microscopic identification, the slide culture method was employed, and observations were conducted using a microscope (Hirox MXB-2500REZ), focusing on characteristics such as hyphal and spore morphology. Data from both microscopic and macroscopic observations were compared against references from books and relevant journals pertaining to the identification of endophytic fungi [22-24].

Cultivation and extraction of endophytic fungi

The fungi were cultivated by placing 6 agar plugs of pure isolates into 300 mL of PDB medium. The culture was incubated for 3 - 4 weeks or until a color change was observed. The mycelium and liquid culture were then separated using filter paper. The mycelium (biomass) was dried at 60 °C and stored in a dry state. The liquid culture was partitioned with ethyl acetate at a 1:1 ratio for 3 days, with daily shaking. The ethyl acetate extract was obtained from the liquid culture using a separating funnel and subsequently concentrated at 40 °C with a rotary evaporator. The concentrated extract was subsequently weighed [25].

Antioxidant activity test

The samples were tested across concentrations from 1,000 down to 15.63 µg/mL. Each endophytic fungal extract (1 mL) was added to 3 mL of DPPH solution (62.5 µM). Following a 30-minute incubation, absorbance was recorded at a wavelength of 517 nm using a spectrophotometer. Ascorbic acid functioned as the positive control, and the inhibition percentage was calculated based on the specified formula [26,27]:

$$\text{Inhibition (\%)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

The percentage inhibition data were used to create a linear regression graph. The IC_{50} value was determined from the linear regression equation $y = ax + b$.

Antibacterial activity test

The antibacterial activity test was conducted using the Kirby-Bauer disk diffusion method. The test bacteria included *Salmonella typhi* (IPCCCB.11.669), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922). Endophytic fungal extracts were applied at a concentration of 400 µg/disk. Negative control disks contained 10 µL of DMSO, while positive control disks were treated with 30 µg of tetracycline. Paper disks were placed on Petri dishes inoculated with the test bacteria, followed by incubation at 37 °C for a duration of 24 h. The inhibition zones were measured in diameter using a caliper. The inhibition zone diameter was evaluated based on established criteria [28]:

Weak: $\frac{A}{B} \times 100\ \% < 50\ \%$

Medium : $50\ \% < \frac{A}{B} \times 100\ \% < 70\ \%$

Strong : $\frac{A}{B} \times 100\ \% > 70\ \%$

Note: A: Sample inhibition zone (mm); B: Antibiotic inhibition zone (mm)

Isolation of pure compounds from selected endophytic fungi

The LM2 extract was analyzed using thin-layer chromatography (TLC) on silica gel G-60 F254 plates with various solvent systems to observe spot patterns. A

total of 2 g of the ethyl acetate extract from endophytic fungus LM2 was pre-adsorbed using silica gel G60 (70 - 230 mesh) at a 1:1 ratio. The extract was then separated through column chromatography using silica gel G60 (70 - 230 mesh; 20 g) as the stationary phase and eluted with a gradient solvent system. The eluate was stored in 10 mL vials, and each fraction was analyzed with TLC to group column fractions based on spot patterns. Spot detection was performed under UV light at λ 254 nm and by spraying with a reagent. Each fraction was evaporated, weighed, transferred to a vial, sealed and stored in a refrigerator. Fractions containing multiple spots underwent further separation using column chromatography with suitable solvents. Fractions with identical TLC retention factors were combined, evaporated, weighed, transferred to vials, sealed and stored in a refrigerator. This process was repeated until fractions with a single spot-on TLC were obtained. The pure fractions were then recrystallized to isolate pure compounds, which were subsequently analyzed using spectroscopy, including 1D and 2D NMR.

Results and discussion

Isolation and identification of endophytic fungi from cashew leaves (*A. occidentale*)

The isolation of endophytic fungi from cashew leaves resulted in the identification of 6 isolates (coded LM1 to LM6). The fungal colonies exhibited a range of macroscopic traits, such as variations in shape and color, along with unique microscopic characteristics (**Figure 1**). The colony colors observed from the cashew leaf samples were predominantly white, gray, black and green. The macroscopic and microscopic characteristics of the endophytic fungal isolates are presented in **Tables 1** and **2**.

Table 1 Colony characteristics of endophytic fungi from Cashew leaves.

Code	Surface colony	Reverse colony	Structure	Elevation	Pattern	Exudate drops	Radial line	Concentric circle
LM1	White to greenish	White	Cottony	Rugose	Zonate	-	-	√
LM2	Green	White	Powder	Umbonate	Spread	-	-	√
LM3	Black	Black	Velvety	Umbonate	Radiate	-	-	-
LM4	Grey	White	Cottony	Umbonate	Zonate	-	-	√
LM5	Grey	Grey	Cottony	umbonate	Spread	-	-	-
LM6	White to greenish	White	Cottony	Rugose	Zonate	-	-	√

Table 2 Microscopic characteristics of endophytic fungi from Cashew leaves.

Isolate	Spore	Shape	Hyphae	Characteristic	Species of Identification
LM1	Sporangia	Subglobose	Septate	The hyphae are in the early phases of growth.	*Trichoderma* sp.
LM2	Conidia	Subglubose	Septate	Conidiophores developed on cushion-shaped structures, hyline erect, branched.	*Trichoderma hamantum*
LM3	Conidia	Subglubose	Septate	Phialides radiate and are biseriate.	*Aspergilus niger*
LM4	Conidia	Glubose	Septate	Conidiospores simple, hyaline, bearing single conidia apically.	*Nigrospora* sp.
LM5	Conidia	Subglubose	Septate	Conidiophores erect, slightly rough.	*Penicillium* sp.
LM6	Conidia	Subglobose	Septate	Conidiophores apically at irregularly.	*Trichoderma pseudokoningii*

Tables 1 and **2** describe the morphological characteristics of endophytic fungal colonies from cashew leaves for each isolate. Six genera of endophytic fungi were identified from the cashew leaves: *Aspergillus* sp. (1 isolate: LM3), *Trichoderma* sp. (3 isolates: LM1, LM2 and LM6), *Nigrospora* sp. (1 isolate: LM4) and *Penicillium* sp. (1 isolate: LM5). Based on the identified morphological traits, both macroscopic and microscopic, 6 endophytic fungal isolates from cashew leaves were identified.

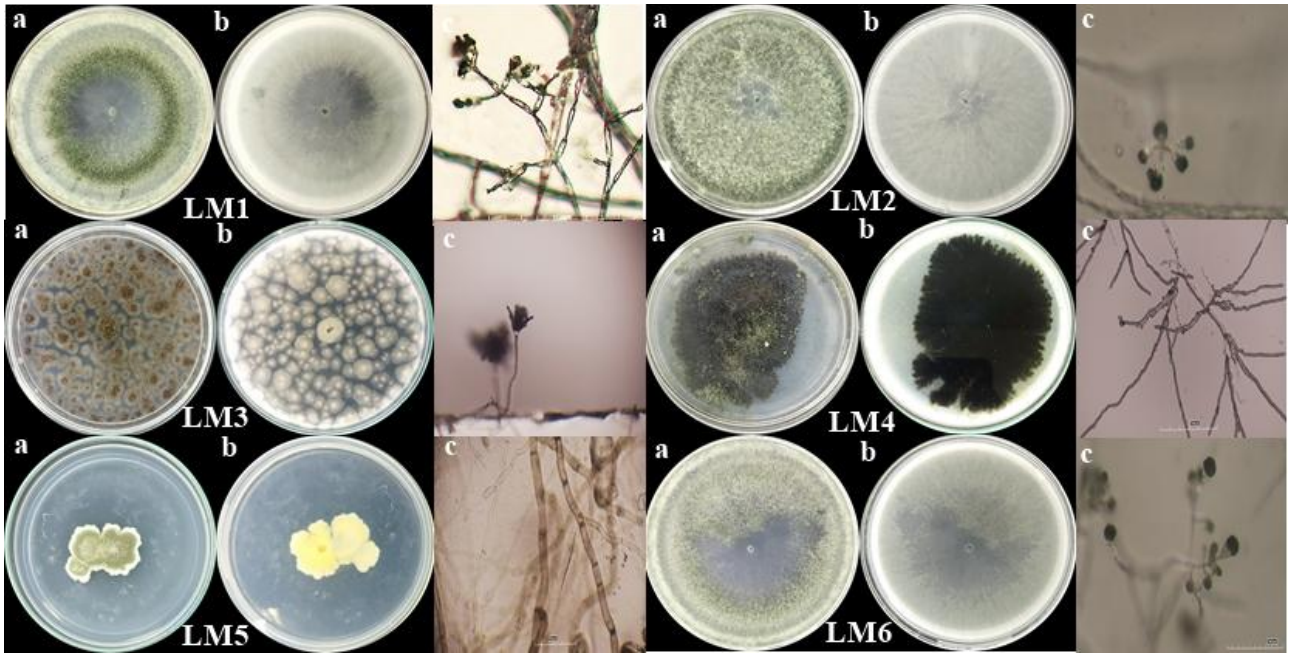

Figure 1 Colony characteristics of endophytic fungi from Cashew leaves (a): Front view; (b): Reverse view; (c): Microscopic view.

Antioxidant and antibacterial activities

Extracts of endophytic fungi from Cashew leaves (Anacardium occidentale)

Endophytic fungi extract from *A. occidentale* leaves, prepared with ethyl acetate solvent, showed promising antibacterial and antioxidant properties (**Table 3**). The tested extracts displayed antimicrobial activity against *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. The majority of the extracts from endophytic fungi exhibited potent antibacterial activity against the tested bacterial strains.

Table 3 Antioxidant and antibacterial activities of endophytic fungal extracts from *A. occidentale* leaves and their host extract.

Sample	Extract	Weight of extract (g/L) of media	% Antibacterial activity				Antioxidant activity IC_{50} (µg/mL)
			E. coli	*S. aureus*	*S. thypi*	*B. subtilis*	
Host plant	Methanol of *A. occidentale* Leave	-	60.1 ± 0.12**	60.3 ± 0.14**	61.3 ± 0.54**	61.9 ± 0.12**	16.816****
Endophytic fungi	LM1	1.1	68.4 ± 0.44**	68.7 ± 0.36**	65.4 ± 0.60**	68.1 ± 0.48**	96.018***
	LM2	4.2	71.6 ± 1.62***	73.1 ± 0.74***	73.6 ± 0.16***	72.9 ± 0.54***	90.117***
	LM3	4.3	60.9 ± 0.64**	59.8 ±1.39**	63.3 ± 0.95**	62.1 ± 0.16**	395.817***

Sample	Extract	Weight of extract (g/L) of media	% Antibacterial activity				Antioxidant activity IC_{50} (µg/mL)
			E. coli	*S. aureus*	*S. thypi*	*B. subtilis*	
	LM4	0.8	80.3 ± 0.18***	81.4 ± 0.40***	81.8 ± 0.16***	80.6 ± 0.68***	40.164***
	LM5	1.5	52.1 ± 0.89**	51.9 ± 0.35**	56.2 ± 0.54**	58.9 ± 0.72**	251.509**
	LM6	1.3	65.3 ± 0.16**	65.4 ± 0.51**	60.1± 0.38**	62.1 ± 1.67**	97.651***
Positive control			Tetracyclin 100***				Ascorbic acid 10.083****

Note: Six endophytic fungal isolates from leaves; LM1 - LM6; Antibacterial activity percentage: *** ≥ 70 % (strong), ** 50 - 70 % (moderate) and ** < 50 % (weak); Antioxidant activity IC_{50} (µg/mL): **** very strong < 20 µg/mL; *** strong < 100 µg/mL; ** moderate 100 - 500 µg/mL; * weak > 500 µg/mL.

Table 3 shows the antibacterial activity of each isolate against various pathogenic strains, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Bacillus subtilis*. The LM4 isolate exhibited the highest activity against all 4 bacterial strains, with inhibition percentages exceeding 80 %, categorizing it as having very strong antibacterial activity. On the other hand, LM2 also demonstrated high antibacterial activity, albeit slightly lower than LM4, but with a larger extract yield.

The antioxidant activity of the isolates also yielded noteworthy results, with LM4 showing an IC_{50} value of 40.164 µg/mL, indicating strong antioxidant activity (IC_{50} category between 20 - 100 µg/mL). This value suggests that the isolate LM4 extract is highly effective in neutralizing free radicals, presenting significant potential for pharmaceutical applications. Isolates LM1 and LM2 also demonstrated antioxidant potential, with IC_{50} values of 96.018 and 90.117 µg/mL, respectively, still classified as strong, though lower than isolate LM4. Isolate LM2 and LM4 exhibited the most promising antibacterial and antioxidant activities, with isolate LM2 producing the highest extract yield and showing potential bioactivity. Morphological identification described isolate LM2 as *Trichoderma* sp.

Trichoderma sp. is well-known for its substantial potential in antibacterial and antioxidant activities, as well as its high biomass production capacity, resulting in abundant extract volume. Naturally, *Trichoderma* sp. produces a range of secondary metabolites, including enzymes such as glucanase and chitinase, as well as phenolic and alkaloid compounds with strong antimicrobial effects against various bacterial pathogens [29-31]. These compounds act by disrupting bacterial cell walls or inhibiting the synthesis of bacterial proteins and DNA, thereby hindering bacterial growth [32-34]. The high antimicrobial activity of *Trichoderma* sp. has been reported in various studies, establishing it as a prime candidate for the development of natural antimicrobial agents [35,36].

Isolation, identification of pure compounds and activity testing

The separation of 2 g of ethyl acetate extract from LM2 using column chromatography (CC) with increasing polarity eluents, namely n-hexane with ethyl acetate (10:0 → 0:10) and ethyl acetate with methanol (10:0 → 5:5 mL), was stored into 72 vials, each containing 10 mL. TLC analysis, employing n-hexane and ethyl acetate in a 5:5 ratio as the mobile phase, identified 5 separate column fractions (F1 to F5). Fraction F4, which displayed a major purple spot under UV light at a wavelength of 254 nm, was selected for further purification through re-chromatography (reCC). TLC analysis resulted in 3 sub-fractions labeled F4.1 - F4.3. Sub-fraction F4.2 was purified by rinsing with a solvent mixture of n-hexane: Ethyl acetate (5:5), yielding compound 1 as yellow crystals (41.4 mg).

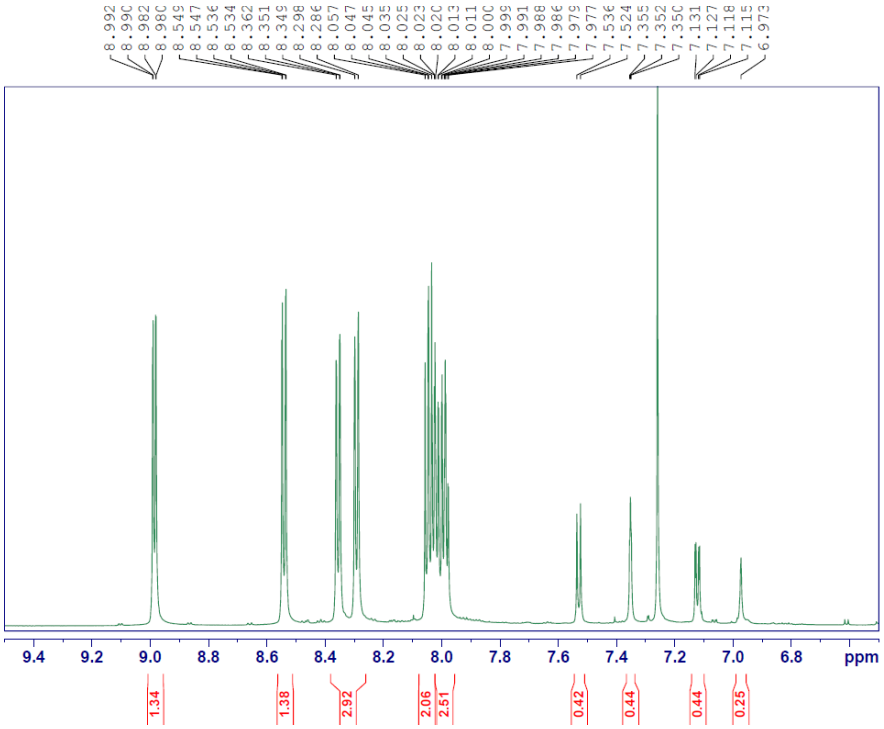

Figure 2 The ^{1}H-NMR Spectrum of Compound 1.

The ^{1}H-NMR spectrum shown in **Figure 2** indicates the presence of 7 aromatic proton signals at δ_H 7.99 (1H, m); 8.02 (1H, m); 8.04 (1H, m); 8.29 (1H, d, 8.4 Hz); 8.35 (1H, d, 7.7 Hz); 8.54 (1H, dd, 1.4 and 9.1 Hz); and 8.99 ppm (1H, dd, 1.4 and 7.0 Hz). The presence of 3 multiplet proton signals suggests that compound 1 contains protons of the AMX type. Two doublet proton signals with different coupling constants indicate that these protons are 3-bond coupled with multiplet protons. Additionally, 2 doublet-doublet proton signals indicate that these protons are also coupled with multiplet protons. This analysis suggests that the 7 aromatic protons are located on 2 aromatic rings.

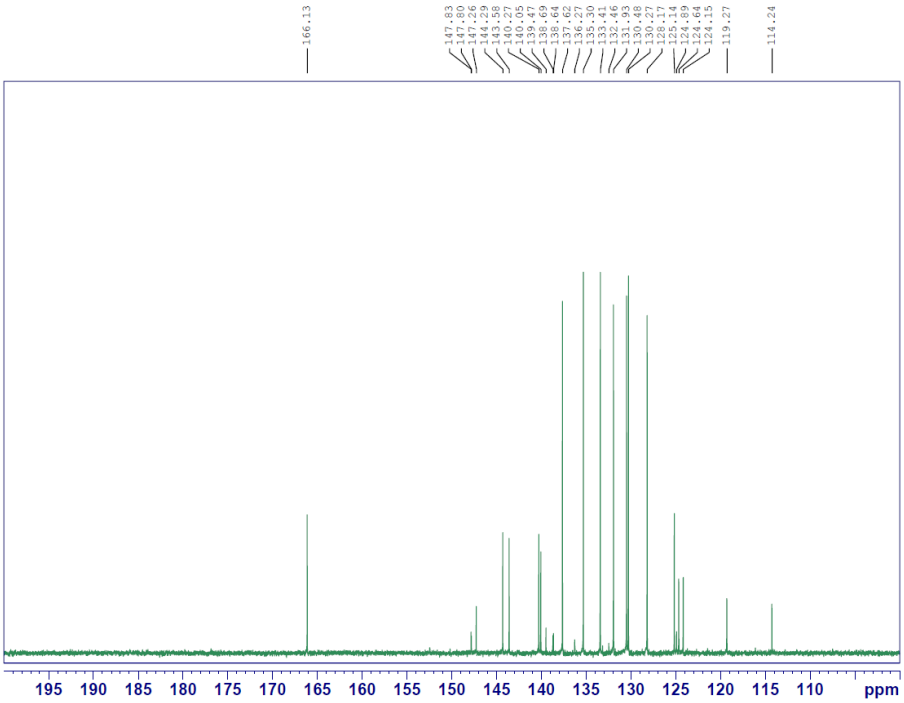

Figure 3 The ^{13}C-NMR spectrum of compound 1.

The ^{13}C-NMR spectrum in **Figure 3** shows 13 carbon signals, including 12 aromatic carbon signals at δ_C 166.1, 144.3, 143.6, 140.3, 140.0, 137.6, 135.3, 133.4, 131.9, 130.5, 130.3, 128.2 and 125.1 ppm, along with a lactone carbonyl signal at δ_C 166.1 ppm. The spectrum also displays 7 carbon signals with high intensity, indicating that compound 1 contains 7 aromatic methine carbons. This data suggests that compound 1 comprises 3 rings: 2 aromatic rings and a 6-membered lactone ring.

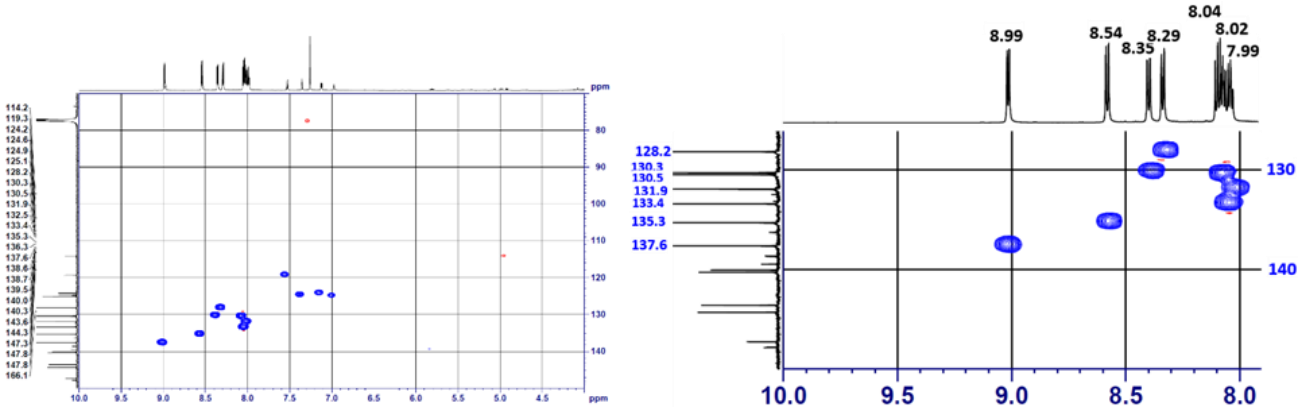

Figure 4 The HSQC spectrum of compound 1.

The HSQC (Heteronuclear Single Quantum Correlation) spectrum in **Figure 4** shows 7 direct correlations between aromatic carbons and 7 aromatic protons. These correlations reinforce the indication from the ^{1}H-NMR spectrum that compound 1 contains 2 aromatic rings, with 3 and 4 aromatic protons, respectively. Four oxyaryl carbons appear at downfield chemical shifts at δ_C 144.3, 143.6, 140.3 and 140.0 ppm, while the lactone carbonyl carbon shows the most downfield shift at δ_C 166.1 ppm. Additionally, 1 oxyaryl carbon is observed at δ_C 125.1 ppm.

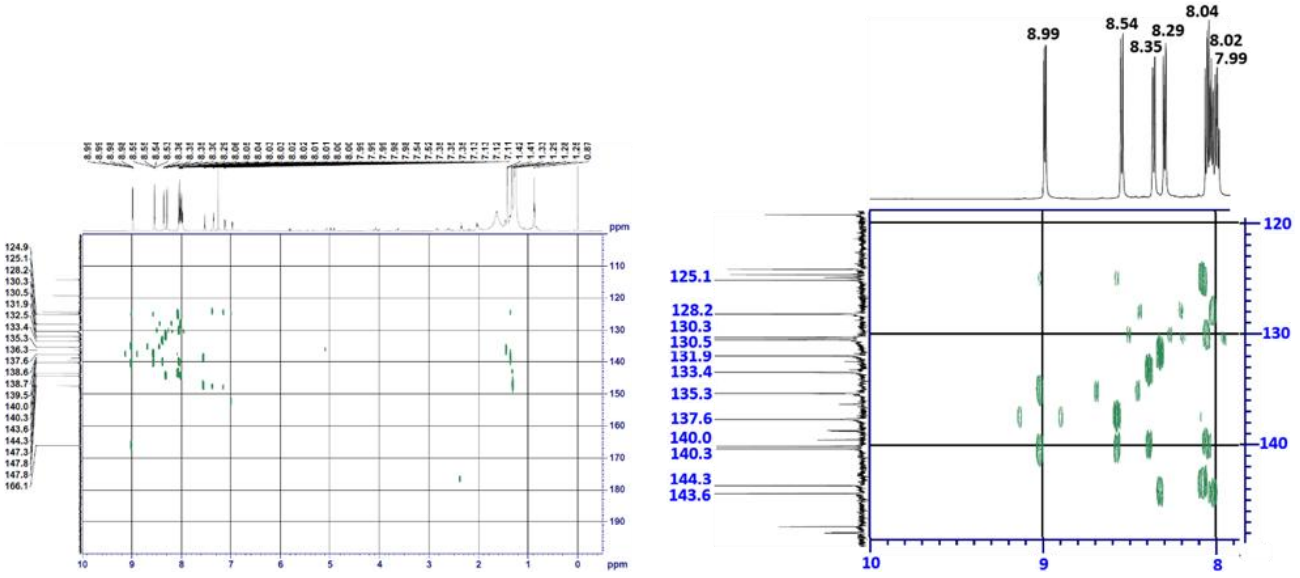

Figure 5 The HMBC spectrum for compound 1.

The correlation between protons and adjacent carbons 2 to 3 bonds away was determined using the HMBC (Heteronuclear Multiple-Bond Correlation) spectrum. The HMBC spectrum of compound 1 (**Figure 5**) shows 2 doublet-doublet proton signals, indicating that these protons are positioned on the same aromatic ring in meta positions. This is evident from the 3-bond correlation between the proton at δ_H 8.99 ppm and

carbons at δ_C 135.3, 140.3 and 125.1 ppm, along with a long-range 4-bond coupling with the carbonyl carbon at δ_C 166.1 ppm. Additionally, the proton at δ_H 8.54 ppm exhibits a 3-bond correlation with carbons at δ_C 125.1 and 137.6 ppm and a 4-bond correlation with the carbon at δ_C 140.3 ppm. Another aromatic proton on the same ring at δ_H 8.04 ppm, showing multiplet multiplicity, correlates 3 bonds away with the carbon at δ_C 144.3 ppm and 4 bonds away with the carbon at δ_C 125.1 ppm.

The HMBC correlation also indicates that the adjacent ring contains 4 aromatic protons. This is supported by the 2 doublet proton signals at δ_H 8.35 ppm, correlating 2 bonds away with carbons at δ_C 133.4 and 140.0 ppm, and the proton at δ_H 8.29 ppm correlating 2 bonds away with carbons at δ_C 131.9 and 143.6 ppm. Further, the 2 multiplet protons at δ_H 8.02 ppm show 2- and 3-bond correlations with carbons at δ_C 130.3 and 140.0 ppm, while the proton at δ_H 7.99 ppm correlates 2 and 3 bonds away with carbons at δ_C 128.2 and 143.6 ppm.

Table 4 1D and 2D NMR spectrum data of compound 1.

No. C	δ_C ppm	δ_H ppm (ΣH. Multiplicity, Hz) 1	HMBC 1
1	130.3	8.35 (1H, d, 7.7)	133.4; 140.0
2	133.4	8.02 (1H, m)	130.3; 140.0
3	131.9	7.99 (1H, m)	128.2; 143.6
4	128.2	8.29 (1H, d, 8.4)	131.9; 143.6
6	166.1		
7	144.3		
8	135.3	8.54 (1H, dd, 1.4 and 9.1)	125.1; 137.6; 140.3
9	130.5	8.04 (1H, m)	125.1; 144.3
10	137.6	8.99 (1H, dd, 1.4 and 7.0)	135.3; 140.3; 166.1; 125.1
11	140.3		
12	125.1		
13	143.6		
14	140.0		

Based on the analysis of the ^{1}H-NMR, ^{13}C-NMR, HSQC and HMBC spectra, compound 1 was found to have 2 aromatic rings, 1 containing 3 aromatic protons and the other containing 4 aromatic protons. A fused 6-membered lactone ring is positioned between these 2 aromatic rings. It is suspected that a hydroxyl group is attached to the aromatic ring adjacent to the lactone carbonyl group, as indicated by the downfield chemical shift of the oxyaryl carbon. However, the hydroxyl proton signal was not observed in the ^{1}H-NMR spectrum. Consequently, compound 1 was identified with the molecular formula $C_{13}H_8O_3$ and a double bond equivalent (DBE) of 10, corresponding to 7-hydroxy-6H-benzo[c]chromen-6-one. The NMR spectral data, both 1D and 2D, for compound 1 are presented in **Table 4**. **Figure 6** illustrates the molecular structure of compound 1, with details on the numbering of carbon atoms, chemical shifts for both proton and carbon and HMBC correlations.

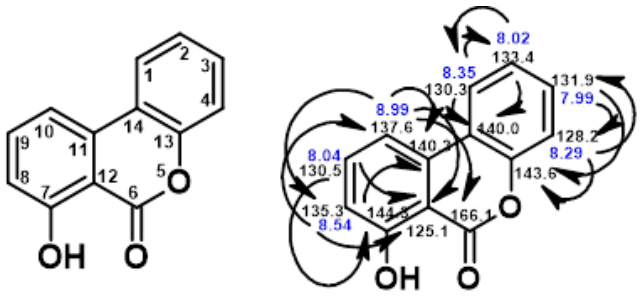

Figure 6 Compound 1 (7-hydroxy-6H-benzo[c]chromen-6-one).

Morphological identification described LM2 as *Trichoderma* sp., also known as a producer of strong natural antioxidant compounds, such as flavonoids and terpenoids, which can counteract free radicals through electron capture mechanisms and oxidative protection [37,38]. This antioxidant capability not only protects cells from oxidative damage but also enhances cellular stability, making it significant for pharmaceutical applications. Previous studies have shown that *Trichoderma* sp. species possess low IC_{50} values in antioxidant activity, indicating a strong ability to neutralize free radicals [39-41]. On the other hand, *Trichoderma* sp. exhibits high growth and biomass production under optimal culture conditions, resulting in a greater extract volume compared to other endophytic fungi [42-44]. This allows for the extraction of abundant bioactive metabolites, which is essential for large-scale production in commercial applications. Following the isolation of a pure compound from the *Trichoderma* sp. extract, compound 1 was identified as a phenolic compound, specifically 7-hydroxy-6H-benzo[c]chromen-6-one (**Figure 6**).

Phenolic compounds, commonly produced by endophytic fungi such as *Trichoderma* sp., have a unique mechanism as antioxidants and antibacterial agents. The primary mechanism of phenolic compounds as antioxidants involves the donation of hydrogen atoms or electrons from the hydroxyl (-OH) group on their aromatic ring to neutralize free radicals [45]. Phenols stabilize free radicals by converting them into less reactive molecules, thereby breaking the chain of oxidative reactions that can damage cells. Oxidation products of phenols, such as phenoxy radicals, are also relatively stable due to the resonance structure of their aromatic ring, preventing these radicals from further reacting with other cellular components. Through this mechanism, phenols protect lipids, proteins, and DNA from oxidative damage, which is beneficial for health and the prevention of degenerative diseases [46-48].

Phenols also exhibit effective antibacterial activity through several mechanisms. First, phenols can disrupt bacterial cell membranes by increasing their permeability, leading to the leakage of ions and essential molecules from within the cell. This disruption impairs membrane function and causes osmotic imbalance, ultimately resulting in bacterial cell death [49-51]. Second, phenolic compounds can inhibit essential bacterial enzymes by interfering with protein and enzyme structures through hydrophobic interactions and hydrogen bonding. Some phenolic compounds are also capable of disrupting DNA and RNA synthesis, thereby inhibiting bacterial replication and genetic expression [52,53]. The combination of these mechanisms makes phenols effective against a a broad spectrum of bacterial pathogens. This dual effect positions phenolic compounds as ideal bioactive agents for creating natural products with antimicrobial and antioxidant properties. The antioxidant and antibacterial activity tests of the pure compound compared to its ethyl acetate extract are presented in **Table 5**. The results shown in Table 5 indicate a decrease in the antioxidant activity of compound 1 as a phenolic compound, alongside a decline in antibacterial effectiveness against 2 bacterial strains, *S. aureus* and *E. coli*. This could result from the combined synergistic effects of multiple components found in the extract, suggesting that the ethyl acetate extract holds more potential for development as a medicinal ingredient compared to the pure compound. Another potential explanation is the presence of other active antioxidant and antibacterial compounds that were not identified or isolated in this study. Nevertheless, compound 1 demonstrated strong

antibacterial activity against 2 bacterial strains *S. typhi* and *B. subtilis*. To develop compound 1 as a medicinal source, structural modification would be necessary, such as substituting a hydroxyl group at C-8 to form a catechol unit in the phenolic compound, it acts as an antioxidant and is also predicted to enhance antibacterial activity.

Table 5 Antioxidant and antibacterial activities of ethyl acetate extract from endophytic fungus LM2 and its pure compound compared to positive control.

Sample	Weight (g)	% Antibacterial Activity				Antioxidant Activity IC_{50} (µg/mL)
		E. coli	*S. aureus*	*S. thypi*	*B. subtilis*	
LM2	2	71.6 ± 1.62***	73.1 ± 0.74***	73.6 ± 0.16***	72.9 ± 0.54***	90.117***
Compound 1	0.0414	55.4 ± 0.97**	57.6 ± 0.82**	70.1 ± 1.05***	71.3 ± 0.71***	> 100[ia]
Positive control		Tetracyclin 100***				Ascorbic acid 10,083****

Note: Antibacterial activity classification: *** ≥ 70 % (strong), **s 50 - 70 % (moderate) and * < 50 % (weak); Antioxidant activity IC_{50} (µg/mL): ia > 100 µg/mL (in active).

Conclusions

This study demonstrated that endophytic fungi isolated from cashew leaves, particularly isolates LM4 and LM2, exhibited significant antibacterial and antioxidant activities. Isolate LM4 showed outstanding efficacy against various pathogens and had a strong antioxidant IC_{50} value, while isolate LM2 yielded the highest extract volume with notable bioactivity. Isolate LM2 was identified as *Trichoderma* sp. that produced diverse secondary metabolites that disrupt bacterial cell walls and protect against oxidative damage. Compound 1, a phenolic derivative specifically identified as 7-hydroxy-6H-benzo[c]chromen-6-one, was isolated from Trichoderma sp. This compound demonstrated a strong antibacterial potential, particularly against *S. typhi* and *B. subtilis*, but showed reduced activity compared to the crude extract, likely due to synergistic effects of other components. Structural modifications, such as adding a hydroxyl group at C-8, could potentially enhance its antioxidant and antibacterial efficacy, making it promising for pharmaceutical applications.

Acknowledgements

The authors express their appreciation to Universitas Sriwijaya, Indonesia for providing financial support for this study through the Skema Penelitian Unggulan Profesi program, in accordance with Rector's Decree Number 0125.026/UN9/SB3.LP2M.PT/2024.

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