

Biological Activities of Endophytic Fungi from Mango Mistletoe (*Dendrophthoe pentandra*) and Tea Mistletoe (*Scurrula atropurpurea*): Antioxidant and Antibacterial Potential

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Abstract. Endophytic fungi represent a valuable source of bioactive compounds, with their metabolite production often shaped by host plants. Notably, endophytic from parasitic plants, such as mistletoe, remain relatively underexplored. This study aimed to isolation and evaluation the antioxidant and antibacterial potential of endophytic fungi associated with mango mistletoe (*Dendrophthoe pentandra*) and tea mistletoe (*Scurrula atropurpurea*). A total of 11 fungal isolates were obtained from mistletoe organs, including leaves, stems, flowers, and petioles. All isolates underwent liquid fermentation for 14–21 days to yield fungal extracts. Antioxidant activity was measured using the DPPH radical scavenging method with ascorbic acid as a standard, while antibacterial effects against *Escherichia coli* were assessed by the disc diffusion method with chloramphenicol as a positive control. Five isolates EFPM 1, EFPM 2, EFPM 3, EEFM, and EFSM 2 exhibited antioxidant activity, though relatively weak compared to ascorbic acid. In contrast, EFSM 2, EFPM 1, EFLT 1 & 2 displayed measurable antibacterial activity, with inhibition zones ranging from 6.26 to 11.95 mm, lower than chloramphenicol. These findings collectively demonstrate the value of bioprospecting endophytic fungi from mango and tea mistletoe, highlighting their potential as a natural source of therapeutic compounds for the discovery of new drugs.

Keywords: antibacterial, antioxidant, endophytic fungi, mango mistletoe, tea mistletoe.

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1. Introduction

Plants are a rich source of biodiversity that have long been used in both traditional and modern medicine due to their ability to produce numerous secondary metabolites. Beyond this wealth of naturally beneficial compounds, plants also interact with various microorganisms that enhance growth, protect against diseases, and support the production of these natural compounds. Among these microorganisms are endophytic fungi [1], which have long attracted the attention of researchers. Endophytic fungi live symbiotically within the cells and intercellular spaces of plant tissues, such as leaves, stems, or roots. This relationship stimulates the fungi to produce bioactive compounds similar to those of their host plants, potentially possessing unique pharmacological properties that are absent in fungi not associated with plants. These metabolite compounds are believed to exhibit biological activities such as anticancer, antiviral, antibacterial, and antioxidant effects, which play crucial roles in human life, particularly in the medical field, and may also enhance plant productivity due to interactions with both pathogenic and non-pathogenic microorganisms.

Currently, most research focuses on plants that are well-known in traditional and modern medicine and are cultivated commercially. However, limited studies have been conducted on the endophytic fungi of mango mistletoe (*Dendrophthoe pentandra*) and tea mistletoe (*Scurrula atropurpurea*) due to their parasitic nature. This accounts for the scarcity of information regarding the hidden benefits offered by these plants, particularly the endophytic fungi associated with them. Both mango and tea mistletoes have been recognized as valuable sources for traditional medicine and have been shown to harbor endophytic fungi in several of their tissues. Previous studies have demonstrated that these mistletoes exhibit anti-inflammatory, antiviral, anticancer, analgesic, antihypertensive [2]; activities, reduce free radicals [3][4] and enhance immunity.

Moreover, extracts of endophytic fungi isolated from mango mistletoe leaves have shown potential as antioxidants and antibacterial agents [2][5][6], suggesting their utility as alternative sources for producing essential secondary metabolites. Nevertheless, information on the potential of endophytic fungi residing in other tissues, such as petioles, leaves, flowers, and stems, of both mango and tea mistletoes remains limited. Therefore, studying endophytic fungi from these mistletoes presents a significant opportunity for discovering novel therapeutic compounds that may have been overlooked in previous research.

Based on the background described, this study aimed to isolate endophytic fungi from various tissues of mango mistletoe (*Dendrophthoe pentandra*) and tea mistletoe (*Scurrula atropurpurea*), and to evaluate the antioxidant and antibacterial activities of their extracts. The study is expected to provide preliminary data on the pharmacological potential of endophytic fungi from these mistletoes, thereby contributing to global efforts in the discovery of novel therapeutic agents.

2. Research Methods

This experimental study was conducted at the Laboratorium Terpadu, Universitas Islam Malang, to isolate endophytic fungi from petioles, leaves, flowers, and stems of mango mistletoe (*Dendrophthoe pentandra*) and tea mistletoe (*Scurrula atropurpurea*). The pharmacological potential of the isolated fungi was evaluated through fermentation, extraction, and assessment of antioxidant and antibacterial activities..

2.1 Isolation, Purification, and Fermentation of Endophytic Fungi

Endophytic fungi were isolated using the direct planting method on Potato Dextrose Agar (PDA) after surface sterilization with 10% NaOCl for 3 minutes [7]. Purification was performed until morphologically distinct pure colonies were obtained. Pure isolates were

fermented by inoculating 1 × 1 cm mycelial plugs into Potato Dextrose Yeast Broth (PDYB) and incubated for 14–21 days under continuous agitation. [2][5][6].

2.2 Extraction of Bioactive Compounds from Endophytic Fungi

After fermentation, biomass and supernatant were separated. The supernatant was extracted with ethyl acetate (1:1 v/v) using liquid–liquid partitioning, while the biomass was macerated with methanol (1:10 w/v) for 24 hours. Both extraction processes were repeated twice. The extracts were concentrated using a rotary evaporator at 40 °C and 100 rpm to obtain crude extracts..

2.3 Antioxidant Activity Assay

Antioxidant activity was determined using the DPPH radical scavenging method. Crude extracts were prepared in 96% ethanol and tested at concentrations of 20–100 µg/mL. Each sample (0.1 mL) was mixed with 3 mL of 0.1 mM DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm, with ascorbic acid as a positive control. Antioxidant activity was expressed as IC₅₀ values calculated from linear regression. Data analysis was performed descriptively by considering absorbance values and the percentage of radical scavenging, calculated as:

$$\text{Radical scavenging (\%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

where:

A₀ = Absorbance of the control

A₁ = Absorbance of the standard/sample

2.4 Antibacterial Activity Assay

Antibacterial activity against *Escherichia coli* was evaluated using the Kirby–Bauer disk diffusion method. Bacterial suspensions were adjusted to 0.5 McFarland and inoculated onto Mueller Hinton Agar [5][6]. Extracts (25–100 ppm) were applied to sterile discs (20 µL). Chloramphenicol (30 µg/disc) and 96% ethanol served as positive and negative controls, respectively. Plates were incubated at 37 °C for 48 hours, and inhibition zones were measured using calipers. Antimicrobial activity was determined by measuring inhibition zones (clear areas surrounding the discs) using calipers, calculated as:

$$\text{Inhibition zone diameter} = \frac{A+B}{2}$$

where:

A = Vertical inhibition zone diameter

B = Horizontal inhibition zone diameter

3. Results and Discussion

3.1 Isolation of endophytic fungi

Isolation of endophytic fungi from different parts of mistletoe plants yielded seven pure isolates from mango mistletoe (*Dendrophthoe pentandra*) and four pure isolates from tea mistletoe (*Scurrula atropurpurea*) (Table 1). Each purified isolate was characterized based on its macroscopic and microscopic morphology [8]. Identification was performed with

reference to the monograph “*Pengenalan Kapang Tropik Umum*” by [9] and “*Illustrated Genera of Imperfect Fungi*”.

In line with previous studies, several of the isolates shared similar morphological characteristics with fungi belonging to the genera *Hormiscium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Penicillium*, *Bipolaris*, *Fusarium*, *Colletotrichum*, and *Sarocladium* [8][10].

Table 1. Code of endophytic fungal isolates successfully obtained

Sources Plant	Isolate code	Sources Plant	Isolate code
Endophytic fungi of petiole mango mistletoe	EFPM 1	Endophytic fungi of flower mango mistletoe	EFFM
	EFPM 2	Endophytic fungi of petiole tea mistletoe	EFPT 1
	EFPM 3		EFPT 2
Endophytic fungi of stem mango mistletoe	EFSM 1	Endophytic fungi of leaf tea mistletoe	EFLT 1
	EFSM 2		EFLT 2

The colony surface of isolate EFPM was initially white (1–3 days old) and later turned black, with a cotton- or wool-like texture, growth zones, zonation, radial furrows, and exudate droplets. These macroscopic features are characteristic of the genus *Hormiscium* [9]. Isolate EFLT 1 exhibited a black, velvety colony with growth zones, zonation, and exudate droplets but lacked radial furrows, resembling the morphology of the genus *Alternaria* [10]. Similarly, isolate EFLT 2 shared similarities with the genus *Penicillium*, showing black to grayish velvety colonies with growth zones, zonation, radial furrows, and exudate droplets [10].

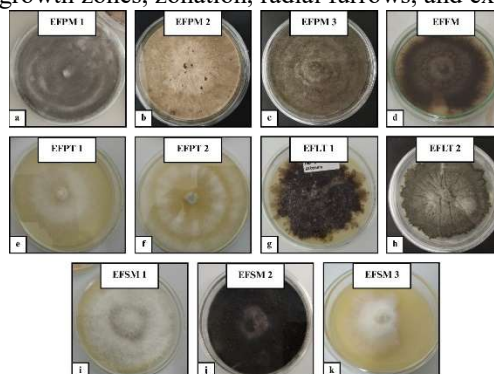


Fig. 1. Macroscopic morphology of endophytic fungal isolates: EFPM 1 (*Hormiscium* spp.) (a), EFPM 2 (*Hormiscium* spp.) (b), EFPM 3 (*Hormiscium* spp.) (c), EFFM (*Bipolaris* spp.) (d), EFPT 1 (*Aspergillus* spp.) (e), EFPT 2 (*Cladosporium* spp.) (f), EFLT 1 (*Alternaria* spp.) (g), EFLT 2 (*Penicillium* spp.) (h), EFSM 1 (*Fusarium* spp.) (i), EFSM 2 (*Sarocladium* spp.) (j), and EFSM 3 (*Colletotrichum* spp.) (k).

Isolate EFPT 1 displayed white colonies with a yellowish to blackish reverse center, cotton-like texture, growth zones, zonation, radial furrows, and distinct exudate droplets, consistent with the genus *Aspergillus* [10]. In contrast, isolate EFPT 2 had white, wool- or cotton-like colonies with a yellow-brown reverse, growth zones, zonation, radial furrows, but without exudate droplets, indicating similarity with the genus *Cladosporium* [10].

Isolate EFSM 1 formed pale whitish-orange, cottony colonies without exudate droplets, radial furrows, or distinct growth zones, although faint zonation was observed. These morphological characteristics are consistent with the genus *Fusarium*. Isolate EFSM 2 exhibited features typical of *Sarocladium*, characterized by initially white colonies that darkened with age, a cottony texture, and the absence of exudate droplets, radial furrows,

zonation, and growth zones. Isolate EFSM 3 produced whitish-orange, cottony colonies with the presence of orange granules at later stages, lacking growth zones, zonation, radial furrows, and exudate droplets, corresponding to the genus *Colletotrichum*. In contrast, isolate EFSM developed dark brown, velvety colonies with rhizoid-like margins, indistinct growth zones, zonation, and exudate droplets, but without radial furrows, suggesting affinity with the genus *Sarocladium*.

All characterized isolates were subjected to submerged fermentation for secondary metabolite production. Fermentation was conducted in liquid media under continuous agitation at ambient temperature for approximately 20 days. The formation of filamentous white structures and yellowish-green to yellowish-brown aggregates in the medium indicated active mycelial growth. The resulting biomass was separated from the culture filtrate, and secondary metabolites were extracted using ethyl acetate, a solvent widely employed for isolating non-polar to semi-polar compounds from endophytic fungal fermentation broths.

3.2 Antioxidant Activity Assay

One of the most widely discussed biological activities in both traditional and modern medicine is the ability of plant-derived compounds to scavenge free radicals, commonly referred to as antioxidant activity. Antioxidant activity reflects the kinetics (rate) of reactions formed between antioxidants and target radical molecules, thereby indicating their ability to block the propagation phase in oxidative chain reactions [11].

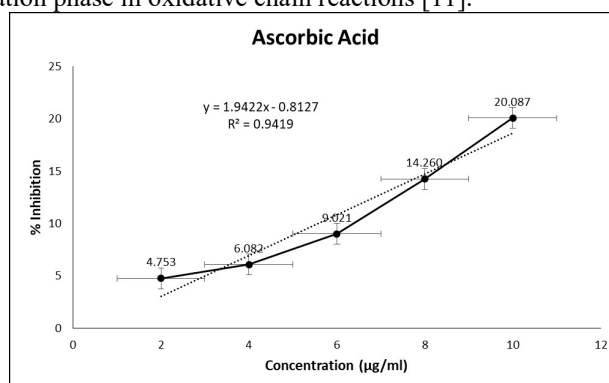


Fig. 2. Standard curve of ascorbic acid as a reference in antioxidant testing

In this study, antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, owing to its simplicity, effectiveness, rapidity, and productivity in reacting with compounds possessing potential antioxidant properties. The results were expressed as IC_{50} values, defined as the concentration required to inhibit 50% of free radicals, with ascorbic acid serving as the positive control [12]. Data were further illustrated as dose–response curves (Figure 2).

Based on IC_{50} classification, a compound is considered a very strong antioxidant if $IC_{50} < 50 \mu\text{g/mL}$, strong if $50 \mu\text{g/mL} < IC_{50} < 100 \mu\text{g/mL}$, moderate if $100 \mu\text{g/mL} < IC_{50} < 150 \mu\text{g/mL}$, weak if $150 \mu\text{g/mL} < IC_{50} < 200 \mu\text{g/mL}$, and very weak if $IC_{50} > 200 \mu\text{g/mL}$.

Ascorbic acid, commonly known as vitamin C, is frequently employed as a reference standard in various phytochemical assays due to its stability, practicality, and well-characterized strong antioxidant properties. Its ability to act as a hydrogen donor and free radical scavenger is particularly effective against DPPH radicals, thereby ensuring the validity and reproducibility of objective and accurate scientific data (Tonthawi, 2023). The stability of ascorbic acid in inhibiting DPPH free radicals was confirmed by a linear regression coefficient of $R^2 = 0.9419$ supporting its suitability as a reliable reference standard.

Table 2. Linear regression equations and IC₅₀ values of endophytic fungi from mango mistletoe and tea mistletoe

Sample	Concentration (µg/ml)	Average % inhibition ± SD	Linear regression	IC ₅₀ (µg/ml)	Antioxidant category
AA*	10	20,087±2,37	$y = 1,9422x - 0,8127$ $R^2 = 0,9419$	26,14	Very strong
	8	14,260±0,87			
	6	9,021±1,15			
	4	6,082±1,15			
	2	4,753±2,00			
EFPM 1	100	6,205±1,83	$y = 0,0556x + 0,9483$ $R^2 = 0,9814$	882,22	Very weak**
	80	5,655±1,19			
	60	4,510±0,71			
	40	3,124±2,76			
	20	1,914±4,69			
EFPM 2	100	7,201±0,36	$y = 0,0541x + 1,3059$ $R^2 = 0,881$	900,08	Very weak**
	80	5,638±1,32			
	60	3,479±0,35			
	40	3,634±0,34			
	20	2,797±0,08			
EFPM 3	100	4,267±2,23	$y = 0,0279x + 1,8511$ $R^2 = 0,8727$	1725,77	Very weak**
	80	4,400±1,52			
	60	3,911±0,93			
	40	2,733±0,57			
	20	2,311±0,91			
EFFM	100	11,390±1,68	$y = 0,113x - 0,1229$ $R^2 = 0,8514$	443,57	Very weak**
	80	10,311±1,09			
	60	4,215±0,50			
	40	4,265±0,38			
	20	3,111±1,59			
EFSM 1	100	6,403±1,21	$y = 0,0084x + 5,5631$ $R^2 = 0,0886$	5290,11	None
	80	6,235±1,12			
	60	6,067±0,56			
	40	5,899±0,81			
	20	5,731±1,39			
EFSM 2	100	5,394±1,56	$y = 0,0438x + 1,3744$ $R^2 = 0,9172$	1110,17	Very weak**
	80	4,934±1,47			
	60	4,473±1,96			
	40	3,443±1,01			
	20	1,762±0,59			
EFSM 3	100	6,545±2,08	$y = 0,0112x + 5,3668$ $R^2 = 0,4178$	3985,11	None
	80	6,572±1,15			
	60	5,318±2,46			
	40	6,081±1,22			
	20	5,672±1,56			
EFLT 1	100	4,704±0,29	$y = 0,0781x - 2,8805$ $R^2 = 0,5017$	2139,37	None
	80	2,491±1,33			
	60	3,723±1,37			
	40	2,918±1,33			
	20	2,239±0,09			
EFLT 2	100	4,186±0,36	$y = -0,0096x + 3,7443$ $R^2 = 0,1996$	4937,95	None
	80	2,450±1,23			
	60	3,037±1,44			
	40	3,446±1,28			
	20	2,731±1,23			
EFPT 1	100	5,319±1,56	$y = -0,0356x + 1,759$ $R^2 = 0,5734$	1355,08	None
	80	3,895±0,46			
	60	4,607±0,98			
	40	3,183±0,78			
	20	2,471±1,21			
EFPT 2	100	7,899±1,82	$y = 0,1176x + 8,489$	686,86	Very weak

Sample	Concentration (µg/ml)	Average % inhibition ± SD	Linear regression	IC ₅₀ (µg/ml)	Antioxidant category
	80	8,501±0,78	R ² = 0,7176		
	60	4,210±1,12			
	40	6,550±2,08			
	20	1,509±0,95			

Description: * AA is ascorbic acid used as a reference
 ** The isolate has better antioxidant properties and valid than other isolates based on linear regression of ascorbic acid R² = 0,9715.

The results demonstrated considerable variation in antioxidant activity among endophytic fungal isolates, indicating that their metabolic capacity does not necessarily mirror that of the host plant, even when isolated from the same tissue. For instance, EFPT 1 and EFPT 2, both obtained from the petioles of *Scurrula atropurpurea*, exhibited different antioxidant potentials. Similarly, among isolates from the petioles of *Dendrophthoe pentandra*, only EFPM 3 showed weak antioxidant activity. In stem-derived isolates, notable activity was observed only in EFSM 2. In contrast, EFLT 1 and EFLT 2 (tea mistletoe petioles) showed no detectable antioxidant activity, whereas EFFM, isolated from mango mistletoe flowers, exhibited strong antioxidant potential.

Although IC₅₀ values reflect differences in antioxidant capacity, they alone are insufficient to evaluate activity reliability. Therefore, the coefficient of determination (R²) was used to assess data validity. As shown in Table 2, several isolates (EFLT 1, EFLT 2, EFSM 3, and EFPT 1) exhibited low R² values, indicating limited data reliability. In contrast, other isolates demonstrated stronger correlations (R² = 0.7–0.9), reflecting more consistent results. Based on the combined assessment of IC₅₀ values (lower values indicating stronger activity) and R², the isolates EFPM 1, EFPM 2, EFPM 3, EFSM 2, and EFFM were identified as having the highest antioxidant potential. Although EFPM 3 showed weaker activity than EFPT 2, its higher R² value suggests greater data reliability and consistency.

3.3 Antibacterial Activity Assay

The antibacterial activity of endophytic fungi from mango (*Dendrophthoe pentandra*) and tea mistletoe (*Scurrula atropurpurea*) was evaluated against the pathogenic bacterium *Escherichia coli* using the Kirby-Bauer disk diffusion method. Chloramphenicol was selected as the positive control due to its broad-spectrum antibiotic activity, effectively targeting both Gram-positive and Gram-negative bacteria. Previous studies have similarly highlighted Chloramphenicol as a standard synthetic antibiotic used in treating bacterial pathogens.

Escherichia coli was chosen because of its complex cell structure and classification as a Gram-negative bacterium, which are naturally more resistant to antibiotics, making it a standard representative for evaluating the efficacy of antibacterial compounds (CLSI, 2020).

Meanwhile, 96% ethanol was used as the negative control to ensure that the solvent itself had no antibacterial effect, confirming that any observed activity was solely attributable to the endophytic fungal extracts. The formation of clear inhibition zones around the discs indicates antibacterial activity, with larger zones reflecting stronger antibacterial potential [13]. As shown in Table 3, several endophytic fungal isolates produced bioactive compounds with antibacterial properties, although their activities varied across isolates and concentrations. Isolate EFSM 2 exhibited antibacterial activity at a minimum concentration of 50 ppm, with the strongest effect observed at 100 ppm. In contrast, EFPM 1 showed detectable activity only at 100 ppm, while EFLT 1 demonstrated optimal antibacterial activity at 75 ppm. Isolate EFLT 2 also displayed antibacterial activity; however, its response did not follow a typical dose–response relationship. Lower concentrations (25 ppm) resulted in stronger inhibition than higher concentrations, indicating a hormetic effect, where a compound elicits different or opposing biological responses depending on its concentration.

[14]. Such non-linear responses are relevant for pharmacological evaluation and dosage optimization.

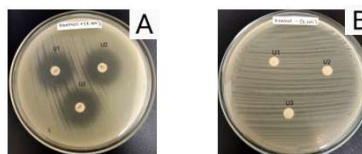


Fig. 3. Controls in the antibacterial test, namely chloramphenicol as a positive control (A) and ethanol as a negative control (B).

The remaining isolates showed no antibacterial activity, which may be attributed to ecological, environmental, geographical, or host-related factors that influence secondary metabolite biosynthesis in endophytic fungi. Overall, the antioxidant and antibacterial assays revealed considerable variability in bioactivity among endophytic fungal isolates from *Dendrophthoe pentandra* and *Scurrula atropurpurea*, even among isolates obtained from the same plant tissues. These differences likely reflect host adaptation, genetic diversity, and environmental conditions that modulate secondary metabolite production in endophytic fungi, including those belonging to the same genus..

Table 3. Results of calculations of the diameter of the inhibition zone in endophytic fungal isolates from mango mistletoe and tea mistletoe.

Sample/Control Extract	Average diameter of the inhibitory zone (mm) $\pm$ SD			
	25 ppm	50 ppm	75 ppm	100 ppm
EFSM 1	-	-	-	-
EFSM 2	-	7,04 $\pm$ 0.60	6.89 $\pm$ 0.34	7,11 $\pm$ 1,01
EFSM 3	-	-	-	-
EFFM 1	-	-	-	-
EFPM 1	-	-	-	6,95 $\pm$ 0,17
EFPM 2	-	-	-	-
EFPM 3	-	-	-	-
EFLT 1	-	-	6,26 $\pm$ 0,24	-
EFLT 2	11,95 $\pm$ 5.98	-	6,17 $\pm$ 0,07	-
EFPT 1	-	-	-	-
EFPT 2	-	-	-	-
K +	21,30 $\pm$ 0,50			
K -	0			

4. Conclusion

The endophytic fungi isolated from mango mistletoe (*Dendrophthoe pentandra*) and tea mistletoe (*Scurrula atropurpurea*) have been characterized and identified as belonging to the genera *Hormiscium*, *Aspergillus*, *Cladosporium*, *Alternaria*, *Penicillium*, *Bipolaris*, *Fusarium*, *Colletotrichum*, and *Sacrocladium*. Several isolates, such as EFPM 1, EFPM 2, EFSM 2, and EFFM, exhibited higher antioxidant activity compared to other isolates, based on the linear regression coefficient (R^2) values and IC_{50} values. Meanwhile, isolates EFSM 2, EFPM 1, and EFLT 1 & 2 demonstrated antibacterial activity against the pathogenic bacterium *Escherichia coli*, with varying inhibition zone diameter.

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