JOURNAL OF TROPICAL PHARMACY AND CHEMISTRY
https://jtpc.ff.unmul.ac.id

Volume 9.2

Research Article

Isolation and Morphological Characterization of Endophytic Fungi from Leaves and Bark of Keledang (*Artocarpus lanceifolius* Roxb.)

Nur Rezky Khairun Nisaa[1*], Amelia Ratih Maharani[2], Rinta Nur Decca Prasanti[2], Putri Anggreini[3], and Leny Eka Tyas Wahyuni[4]

[1]Department of Pharmacognosy-Medicinal Chemistry, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, Indonesia
[2]Study Program of Pharmacy, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, Indonesia
[3]Department of Pharmacology, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, Indonesia.
[4]Bachelor of Nutrition Study Program, Faculty of Public Health, Universitas Mulawarman, Samarinda, Indonesia.
*Correspondence email : nrkhairunnisaa77@farmasi.unmul.ac.id

Abstract

Artocarpus lanceifolius Roxb which is also known as Keledang., an endemic member of the *Moraceae* family, exhibits notable pharmacological activities, including anti-inflammatory, antibacterial, antioxidant, and photoprotective effects associated with its secondary metabolites. Endophytic fungi, which inhabit plant tissues asymptomatically, are known to synthesize bioactive compounds comparable to those of their host plants. This integrated study aimed to isolate and characterize endophytic fungi from the leaves and bark of A. *lanceifolius* to assess their potential as alternative sources of bioactive metabolites. Leaf and bark samples were surface sterilized, sectioned into explants, and inoculated onto Potato Dextrose Agar (PDA). Emerging colonies were purified and examined macroscopically based on colony color, texture, margin, and reverse pigmentation, and microscopically based on hyphal morphology, conidiophores, phialides, and conidia. As an exploratory descriptive study, no statistical tests were applied. Eight endophytic fungal isolates were obtained: four from leaves—*Gliocladium* sp., *Geotrichum* sp., and two *Aspergillus* spp. and three from bark *Penicillium* sp., *Fusarium* sp., and *Aspergillus niger*. The differences between leaf and bark-derived isolates indicate organ-specific ecological niches. These findings demonstrate that *A. lanceifolius* hosts diverse endophytes with potential for natural product discovery, warranting further molecular and metabolomic investigation.

Keywords: *Artocarpus lanceifolius* Roxb, Keledang, Endophytic fungi, Isolation.

Accepted: 22 September 2025
Approved: 20 Oktober 2025
Publication: 16 November 2025

Citation :
N.R.K. Nisaa, A.R. Maharani, R.N.D. Prasanti, P. Anggreini, and L.E.T.Wahyuni, "Isolation and Morphological Characterization of Endophytic Fungi from Leaves and Bark of Keledang (*Artocarpus lanceifolius* Roxb.)", Journal of Tropical Pharmacy and Chemistry (JTPC), Vol. 9.2, pp. 186-190, Nov. 2025, doi: 10.30872/jtpc.v9i2.305

 Doi: 10.30872/jtpc.v9i2.305

1 Introduction

Endophytic fungi are microorganisms that live within healthy plant tissues without causing apparent disease [1]. These organisms form complex symbiotic relationships with their host plants, often contributing to the production of secondary metabolites with significant pharmacological potential [1]. Worldwide, endophytic fungi have been widely studied as alternative and sustainable sources of bioactive compounds, including antimicrobials, antioxidants, anticancer agents, and enzyme inhibitors [2], [3]. Their ability to biosynthesize metabolites like those produced by their host plants makes endophytic fungi attractive candidates for natural product discovery and pharmaceutical development [4], [5].

Artocarpus lanceifolius Roxb., commonly known as Keledang, is an endemic species belonging to the family *Moraceae* and distributed in specific regions of Southeast Asia, including Kalimantan, Indonesia [6]. Traditionally, various parts of *A. lanceifolius* have been used for medicinal purposes. Recent phytochemical and pharmacological investigations have reported that this plant possesses anti-inflammatory, antibacterial, antioxidant, and photoprotective activities. These therapeutic properties are closely associated with their diverse secondary metabolites, such as phenolics, flavonoids, and terpenoids [7], [8].

In addition to the plant's inherent bioactivity, the presence of endophytic fungi within its tissues may also contribute to its pharmacological potential [6]. Since many endophytes can produce metabolites that mimic those of their host plants, the exploration of endophytic fungi from *A. lanceifolius* may uncover novel species or new strains capable of biosynthesizing medically relevant compounds [9]. Despite the promising pharmacological profile of the Keledang plant, studies on its associated endophytic fungi remain limited, particularly in terms of diversity, taxonomic identity, and morphological characteristics [6], [10].

Leaf and bark tissues represent two distinct microenvironments within a plant, each capable of supporting different endophytic communities [11]. Leaves are directly exposed to external environmental stressors, whereas bark tissues serve as protective structures that may harbour unique fungal populations [12]. Investigating both tissues simultaneously provides a more comprehensive understanding of the plant's endophytic diversity and enhances the likelihood of discovering taxonomically and functionally important species [13].

This integrated study aims to isolate, purify, and characterize endophytic fungi from the leaves and bark of *Artocarpus lanceifolius* Roxb. using macroscopic and microscopic morphological analyses. By combining findings from both plant organs, this research provides a broader perspective on endophytic diversity and highlights potential fungal genera with relevance to natural product development. The results also serve as foundational data for subsequent biochemical or molecular studies that may explore the bioactive potential of these isolates.

2 Method

The materials used in this study included leaves and bark of Keledang collected from Sanga-Sanga District, Potato Dextrose Agar (PDA) medium, chloramphenicol solution (150 mg/L), 70% ethanol, 5.25% sodium hypochlorite (NaOCl), sterile distilled water, and cotton. The equipment used consisted of an autoclave, Laminar Air Flow cabinet, hot plate (stirrer), Petri dishes, spirit lamp, forceps, scalpel handle, surgical blades no. 11, dissecting scissors, Erlenmeyer flasks, spatula, aluminium foil, wrapping materials, and binocular microscope.

Leaf and bark samples of Artocarpus lanceifolius were collected and thoroughly washed under running water, after which the plant parts were separated. Surface sterilization was performed following a modified procedure described by Roopa [14]. After air-drying, the tissues were aseptically cut into small segments measuring approximately 1 × 1 mm. The sterilized segments were then transferred onto Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 23 ± 2°C. Fungal growth was monitored periodically, and emerging colonies were subsequently purified to obtain distinct endophytic fungal isolates.

Doi: 10.30872/jtpc.v9i2.305

3 Result and Discussion

The findings from both the leaf and bark of *Artocarpus lanceifolius* Roxb. demonstrate that these plant tissues harbor diverse endophytic fungi. Research utilizing endophytic fungi is highly valuable in the pharmaceutical field due to their ability to produce bioactive compounds. A major advantage of endophytic fungi is their capacity to be cultivated on a large scale without relying on rare or slow-growing plant sources. Exploring endophytic fungi from the bark of *A. lanceifolius* represents an important initial step in identifying alternative producers of secondary metabolites without the need to harvest the host plant. Considering that *A. lanceifolius* is an endemic species with relatively slow and seasonal growth, such an approach supports the conservation of the plant while enabling continued access to its potential bioactive constituents.

Figure 1. Leaves and bark of Keledang

Four endophytic fungal isolates were successfully obtained from the leaves of *Artocarpus lanceifolius* Roxb., designated as DK_1, DK_2, DK_3, and DK_4 (Figure 2). These isolates exhibited distinct morphological characteristics and were tentatively identified as belonging to the genera *Gliocladium sp.*, *Geotrichum sp.*, and *Aspergillus spp*. Meanwhile, four endophytic fungi were also isolated from the bark of *A. lanceifolius*. Isolate KB1 (Bark 1), characterized by a dark-green colony with smooth white margins, was presumed to be *Penicillium sp.* Isolate KB2 (Bark 2), forming black-spotted colonies, corresponded to *Aspergillus niger*. Isolate KB3 (Bark 3), which produced white colonies, was suspected to be *Fusarium sp*

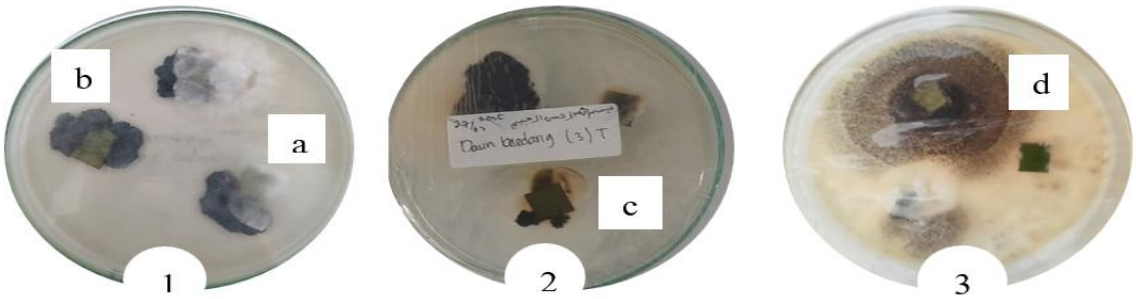

Figure 2. Results of endophytic fungi of keledang leaves (*Artocarpus lanceifolius* Roxb). (a) Isolate DK_1, (b) Isolate DK_2, (c) Isolate DK_3, (d) Isolate DK_4,

Macroscopically, isolate DK_1 exhibited white colonies on the surface and pale greenish-white coloration on the reverse. The colony displayed an irregular circular shape with a slightly depressed center, coarse texture, uneven elevation, and a visible growing zone. The colony reached a diameter of 2.29 cm on day 5 of incubation. Microscopically, DK_1 showed asexual spores in the form of conidia borne

Doi: 10.30872/jtpc.v9i2.305

at the tips of conidiophores, characterized by a rounded shape. The hyphae were septate and hyaline, lacking any pigmentation.

Macroscopically, isolate DK_2 presented black colonies on both the surface and reverse sides. The colony had a coarse texture, irregular circular shape, uneven elevation, and no observable growing zone. After 5 days of incubation, the colony diameter measured 2.33 cm. Microscopically, DK_2 produced asexual spores in the form of arthrospores with a rod-like (rectangular) morphology. The hyphae were darkly pigmented, appearing greenish-black, and septation was clearly observed.

Macroscopically, isolate DK_3 displayed white colonies in the central surface region with orange pigmentation at the margins and on the reverse. The colony had a coarse texture, irregular circular morphology, and uneven elevation, with no visible growing zone. DK_3 grew relatively slower than the other isolates, reaching a diameter of 2.57 cm after 5 days of incubation. Microscopically, DK_3 exhibited asexual spores in the form of conidia that were round and greenish-black. The hyphae were septate, and the long, unbranched conidiophores were hyaline. The isolate possessed a spherical vesicle at the tip of the conidiophore, upon which conidia were arranged.

Macroscopically, isolate DK_4 formed black, spot-like colonies on the surface with a grayish coloration on the reverse. The colony exhibited raised, radially spreading elevation, coarse texture, irregular circular shape, and a clearly visible growing zone. DK_4 grew relatively rapidly, reaching a diameter of 2.82 cm on day 5. Microscopically, DK_4 produced asexual spores in the form of round, greenish-black conidia. The hyphae were septate and hyaline, and the long, unbranched conidiophores were also hyaline. A spherical vesicle bearing clustered conidia was observed at the tip of each conidiophore.

Figure 3. Results of endophytic fungi from the bark of the keledang tree (*Artocarpus lanceifolius* Roxb). (a) Isolate KB_1, (b) Isolate KB_2, (c) Isolate KB_3.

The content Macroscopically, isolate KB1 displayed dark green colonies with a white reverse surface. The colony had a powdery texture, forming irregular circular or oval patterns with uneven elevation. Microscopically, KB1 produced clear, spherical conidia as its asexual spores. During observation, the hyphal structure was not clearly visible. This could be due to tissue damage during preparation or sampling, as the hyphal structure is delicate and thin. Furthermore, thin hyphae have difficulty absorbing dyes properly, making methylene blue staining difficult to see clearly under a microscope.

Macroscopically, isolate KB2 formed black colonies with a white reverse surface. The colony exhibited a powdery texture, irregular circular pattern, and uneven elevation. Microscopically, the fungus showed septate, hyaline hyphae and produced spherical conidia arranged irregularly. This identification is supported by the presence of upright conidiophores and round conidia which are characteristic of asexual spores, while macroscopically, the black color of the colonies with irregular growth patterns is in accordance with the description of the genus.

Macroscopically, isolate KB3 developed white colonies with a regular circular shape and fine cotton-like or wool-like texture. Microscopically, KB3 did not show clearly distinguishable reproductive

Doi: 10.30872/jtpc.v9i2.305

structures; only septate, hyaline hyphae were observed, and conidia were not detected. Based on the results of macroscopic observations showing white colonies with a cotton-like texture and a regular round colony shape, isolate KB3 has similarities with the characteristics of the genus Fusarium.

Table 1. Macroscopic and Microscopic Characteristics of Endophytic Fungal Isolates

Isolate Code	Color	Shape	Texture	Elevation	Reverse Color	Hyphae	Spore Type	Conidia Shape
DK1	White	Round, irregular, depressed center	Coarse	Uneven	Pale greenish-white	Septate, hyaline	Conidia	Spherical
DK2	Black	Round, irregular	Coarse	Uneven	Black	Septate, dark	Arthrospores	Rod/ rectangular
DK3	White with orange margin	Round, irregular	Coarse	Uneven	Orange	Septate, hyaline	Conidia	Spherical
DK4	Black	Round, irregular	Coarse, dry	Raised	Gray	Septate, hyaline, filamentous	Conidia	Spherical
KB1	Dark green	Round, irregular	Powdery	Uneven	White	Not visible	Conidia	Spherical
KB2	Black	Round, irregular	Powdery	Uneven	White with black edge	Septate, hyaline	Conidia	Spherical
KB3	White	Round, regular	Cotton-like	Uneven	White	Septate, hyaline	Not visible	Not visible
KB4	White → gray-black	Round, irregular	Cotton-like / hairy	Uneven	Black base	Septate, long	Conidia	Dark spherical

This study has several notable limitations across methodological aspects. From a methodological standpoint, species-level identification of the fungal isolates could not be confirmed definitively because molecular analyses, which represent the gold standard for species determination, were not performed. At present, identification relies solely on morphological characteristics, meaning that the possibility of other strains with similar morphological traits cannot be excluded.

4. Conclusion

This study demonstrated that the leaves and bark of *Artocarpus lanceifolius* Roxb. harbor diverse endophytic fungi with distinct macroscopic and microscopic characteristics. A total of seven isolates representing multiple genera including *Gliocladium*, *Geotrichum*, *Aspergillus*, *Penicillium*, and *Fusarium*—were successfully identified based on morphological traits. The variation in colony color, texture, hyphal structure, and conidial morphology suggests that different plant organs provide unique ecological niches for fungal colonization. Overall, this research establishes a foundational understanding of the endophytic fungal diversity in *A. lanceifolius* and supports further investigation into their pharmaceutical relevance.

5. Declarations

5.1 Acknowledgements

We would like to thank the Pharmacy Education and Research Laboratory of the Faculty of Pharmacy, Mulawarman University for all the support in carrying out the work.

5.2 Author contributions

The names of the authors listed in this journal contributed to this research.

5.3 Ethics
This research does not require a code of ethics so it does not have a code of ethics.

5.4 Conflict of Interest
The authors declare that they have no conflict of interests.

5.5 Funding Statement

6. Bibliography
[1] G. M. Mohammed and S. N. Hawar, "Morphological and Molecular Characterisation of Endophytic Fungi Isolated from Moringa oleifera Leaves in Iraq and Chemical Analysis of Leaves Extracts Using GC-Mass," *Asian J. Water, Environ. Pollut.*, vol. 21, no. 1, pp. 63–70, Feb. 2024, doi: 10.3233/AJW240009.
[2] L. Al Husnain, L. Alajlan, M. D. F. AlKahtani, R. orfali, and F. Ameen, "Avicennia marina endophytic fungi shows antagonism against tomato pathogenic fungi," *J. Saudi Soc. Agric. Sci.*, vol. 22, no. 4, pp. 214–222, May 2023, doi: 10.1016/j.jssas.2022.12.001.
[3] R. Oktiansyah, H. Widjajanti, A. Setiawan, and Elfita, "Antioxidant and Antibacterial Activity of Endophytic Fungi Isolated from Fruit of Sungkai (Peronema canescens)," *Sci. Technol. Indones.*, vol. 9, no. 1, pp. 17–27, Jan. 2024, doi: 10.26554/sti.2024.9.1.17-27.
[4] X. Ji, Y. Xia, H. Zhang, and J. L. Cui, "The microscopic mechanism between endophytic fungi and host plants: From recognition to building stable mutually beneficial relationships," Aug. 01, 2022, *Elsevier GmbH*. doi: 10.1016/j.micres.2022.127056.
[5] W. Zuo *et al.*, "Molecular Interactions between Smut Fungi and Their Host Plants," Aug. 25, 2019, *Annual Reviews Inc.* doi: 10.1146/annurev-phyto-082718-100139.
[6] R. S. Dewi, H. Hardiansyah, and M. Mahrudin, "Keanekaragaman Jenis Artocarpus di Bantaran Sungai Desa Beringin Kecana Kecamatan Tabunganen Kalimantan Selatan," *Wahana-Bio J. Biol. dan Pembelajarannya*, vol. 13, no. 2, p. 124, Nov. 2021, doi: 10.20527/wb.v13i2.12058.
[7] N. Hidayah, D. Daniel, and E. Marliana, "Aktivitas Ekstrak Metanol Daun Keledang (Artocarpus Lanceifolius Roxb) Sebagai Antiinflamasi," *Pros. Semin. Nas. Kim.*, pp. 126–131, 2021.
[8] H. Hasan, R. Armus, Madania, and I. Marzuki, "Chemical Components of Artocarpus lanceifolius Roxb and Antioxidant Activity," in *AIP Conference Proceedings*, American Institute of Physics Inc., Jan. 2023. doi: 10.1063/5.0112313.
[9] A. Sharma, B. Malhotra, H. Kharkwal, G. T. Kulkarni, and N. Kaushik, "Therapeutic agents from endophytes harbored in Asian medicinal plants," Jun. 01, 2020, *Springer*. doi: 10.1007/s11101-020-09683-8.
[10] E. J. Rivana, "Keanekaragaman Senyawa Flavonoid Terprenilasi Dari Tumbuhan Keledang (Artocarpus Lanceifolius Roxb) Serta Aktivitas Sitotoksinya Terhadap Sel Murine Leukemia p-288," *Univ. Pendidik. Indones.*, p. 14, 2020.
[11] J. Alonso-Serra *et al.*, "Tissue-specific study across the stem reveals the chemistry and transcriptome dynamics of birch bark," *New Phytol.*, vol. 222, no. 4, 2019, doi: 10.1111/nph.15725.
[12] J. Mano, "Early events in environmental stresses in plants - induction mechanisms of oxidative stress," in *Oxidative stress in plants*, 2002.
[13] M. A. Fritz, S. Rosa, and A. Sicard, "Mechanisms Underlying the Environmentally Induced Plasticity of Leaf Morphology," 2018. doi: 10.3389/fgene.2018.00478.
[14] G. Roopa *et al.*, "Identification of Taxol-producing endophytic fungi isolated from Salacia oblonga through genomic mining approach," *J. Genet. Eng. Biotechnol.*, vol. 13, no. 2, 2015, doi: 10.1016/j.jgeb.2015.09.002.