Jurnal Farmasi Sains dan Obat Tradisional
Pharmacy Science and Traditional Medicine Journal
OPEN ACCESS
Published by
LPPM Academy of Pharmacy Imam Bonjol Bukittinggi
E-ISSN 2830-4802
Available online at https://ejournal.akfarimambonjol.ac.id

POTENSI ANTIBAKTERI JAMUR ENDOFIT YANG DIISOLASI DARI *Pachyrhizus erosus* (BENGKOANG)

Khiky Dwinatrana[1*], Lira Fadilla [2], Rustini Rustini [3], Arif Ferdian[1]
[1] Program Studi Farmasi Klinis, Fakultas Ilmu Kesehatan, Universitas Baiturrahmah, Padang, Sumatera Barat
[2] Program Studi Farmasi, Fakultas Farmasi Sains dan Teknologi, Universitas Dharma Andalas, Padang, Sumatera Barat
[3] Fakultas Farmasi, Universitas Andalas, Padang Sumatera Barat

*Email Korespondensi : khikynatrana@fikes.unbrah.ac.id

ABSTRAK

Pencarian agen antibakteri baru semakin banyak difokuskan pada jamur endofit yang hidup di jaringan tumbuhan dan dapat melengkapi aktivitas bioaktif inangnya. *Pachyrhizus erosus* (bengkuang), suatu legum pangan yang kaya fenolik dan flavonoid, masih jarang dieksplorasi sebagai sumber jamur endofit dengan potensi antibakteri. Penelitian ini bertujuan untuk mengisolasi jamur endofit dari *P. erosus* serta mengevaluasi aktivitas antibakterinya. Jaringan umbi dan daun disterilisasi permukaan, menghasilkan lima isolat (PE1–PE5) yang kemudian dikultur pada media beras, diekstraksi dengan etil asetat, dan diuji terhadap *Staphylococcus aureus* ATCC 29213 dan *Escherichia coli* ATCC 25922 menggunakan metode difusi cakram Kirby–Bauer. Seluruh ekstrak menunjukkan aktivitas penghambatan, dengan isolat PE2 memberikan efek paling kuat (15,7 ± 1,40 mm terhadap *S. aureus* dan 14,4 ± 0,32 mm terhadap *E. coli).* Profiling KLT ekstrak PE2 menunjukkan beberapa bercak di bawah UV (254 dan 366 nm), sementara uji fitokimia mengindikasikan adanya fenolik dan triterpenoid. Secara keseluruhan, hasil penelitian ini menunjukkan bahwa jamur endofit yang berasosiasi dengan umbi *P. erosus* mampu menghasilkan metabolit antibakteri yang sebanding dengan senyawa asal tanaman, sehingga berpotensi dikembangkan sebagai sumber agen antimikroba.

Kata kunci : *Pachyrhizus erosus*, Jamur endofit, Aktivitas antibakteri, Metabolit sekunder, Kromatografi Lapis Tipis (KLT).

ANTIBACTERIAL POTENTIAL OF ENDOPHYTIC FUNGI ISOLATED FROM *Pachyrhizus erosus* (BENGKOANG)

ABSTRACT

The search for new antibacterial agents has increasingly focused on endophytic fungi, which inhabit plant tissues and may complement the bioactivity of their hosts. Pachyrhizus erosus (bengkoang), an edible legume rich in phenolics and flavonoids, remains underexplored as a reservoir of fungal endophytes with antibacterial potential. This study aimed to isolate endophytic fungi from P. erosus and evaluate their antibacterial properties. Tuber and leaf tissues were surface sterilized, yielding five isolates (PE1–PE5) that were cultured on rice medium, extracted with ethyl acetate, and tested against Staphylococcus aureus ATCC 29213 and Escherichia coli ATCC 25922 using the Kirby–Bauer disc diffusion method. All extracts inhibited bacterial growth, with PE2 showing the strongest activity (15.7 ± 1.40 mm for S. aureus and 14.4 ± 0.32 mm for E. coli). TLC profiling of PE2 extract revealed multiple spots under UV (254 and 366 nm), and phytochemical screening indicated the presence of phenolics and triterpenoids. Taken together, the results demonstrate that tuber-associated endophytic fungi of P. erosus are capable of producing antibacterial metabolites comparable to plant-derived compounds, underscoring their potential as sources of antimicrobial agents.

Keywords : *Pachyrhizus erosus, Endophytic fungi, Antibacterial activity, Secondary metabolite, Thin-Layer Chromatography (TLC).*

INTRODUCTION

Bacterial infections continue to be a pressing global health challenge. According to the World Health Organization (WHO), *Staphylococcus aureus* and *Escherichia coli* are among the most clinically significant pathogens, frequently associated with community and hospital-acquired infections worldwide. The rise of antimicrobial resistance (AMR) has further heightened concerns, as resistant strains such as methicillin-resistant *S. aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing *E. coli* are increasingly difficult to treat (WHO, 2023). While this study does not focus on resistant isolates, standard laboratory strains of *S. aureus* and *E. coli* remain valuable models for preliminary antibacterial screening, serving as the first step in evaluating novel natural product candidates before clinical testing.

Natural products remain indispensable in antibacterial drug discovery, yet underutilized edible crops and their microbial symbionts are often overlooked. *Pachyrhizus erosus* (bengkoang/jicama), a widely consumed leguminous tuber, has been

shown to contain diverse secondary metabolites such as phenolic acids, flavonoids, and isoflavonoids. These compounds have been associated with antimicrobial, antioxidant, and antibiofilm activities (Jaiswal et al., 2021; Xiang et al., 2025; Oni et al., 2025). Several pure natural products have been reported from *Pachyrhizus erosus*, notably the isoflavones daidzein and genistein, as well as various triterpenoids. These compound classes are known to exhibit antimicrobial activity in vitro, suggesting that both plant extracts and their associated endophytic fungi may be promising sources of antibacterial agents (Jaiswal et al., 2021). Despite these reports, studies investigating the role of endophytic fungi associated with *P. erosus* as a source of antibacterial metabolites remain scarce.

Endophytic fungi have emerged as prolific producers of structurally diverse bioactive compounds. Several recent reviews and experimental studies highlight endophyte-derived metabolites such as polyketides, alkaloids, and peptides with potent antibacterial activity against both Gram-positive and Gram-negative bacteria (Ortega et al., 2025; Nazir et al., 2024; Rosdee et al., 2025). Importantly, endophytes are capable of producing unique metabolites influenced by their host plant microenvironment, often yielding novel scaffolds distinct from those of soil fungi. In this context, the present study aims to screen ethyl acetate extracts of endophytic fungi isolated from the leaf and tuber tissues of *P. erosus* for antibacterial activity against *S. aureus* and *E. coli*. This work addresses an exploratory gap in endophyte research on edible legumes and provides preliminary evidence for the potential antibacterial applications of *P. erosus*-associated fungi.

MATERIAL AND METHODS

Material

Plant material used in this study was *Pachyrhizus erosus* (L.) Urb. (Bengkuang), collected from Olo Gadut, Padang City, Indonesia. The specimen was taxonomically identified at the Herbarium of Andalas University, Padang, Indonesia, and deposited under voucher number 386/K-ID/ANDA/V/2024. Test organisms were *Staphylococcus aureus* (ATCC 25922) and *Escherichia coli* (ATCC 29213). Sabouraud Dextrose Agar (SDA), Nutrient Agar (NA), chloramphenicol, ethyl acetate, ethanol 70%, sodium hypochlorite (NaOCl), rice for solid-state cultivation, and all other reagents were obtained as stated in the laboratory inventory.

Methods

Surface sterilization and isolation of endophytic fungi

Plant samples (leaf and tuber) were processed separately. Samples were rinsed under running tap water to remove debris, followed by rinsing with sterile distilled water. Surface sterilization was performed sequentially as follows: immersion in 70% ethanol for 1 min, rinsing with sterile distilled water, immersion in 2.5% NaOCl for an appropriate time, and final rinsing three times with sterile distilled water. The last wash was plated on SDA as a negative control to verify sterilization efficacy. After sterilization, tissues were aseptically cut into small pieces, homogenized in sterile distilled water, and serially diluted to 10^{-6}. Aliquots (1 mL) of appropriate dilutions were spread onto SDA plates supplemented with chloramphenicol (50 mg L^{-1}) to suppress bacterial contaminants. Plates were incubated at room temperature for 6 days, and colonies with distinct morphology were selected for purification (Kjer et al., 2010; Sandrawati et al., 2023).

Purification and macroscopic characterization of endophytic fungi

Emerging fungal colonies were purified by sub-culturing single colonies onto fresh SDA until morphologically pure isolates were obtained. Purified isolates were described macroscopically (colony colour, texture, margin) and preserved on SDA slants (Kjer et al., 2010; Sandrawati et al., 2023).

Cultivation of endophytic fungi

For metabolite production, each pure isolate was transferred aseptically onto sterile rice medium. Rice medium was prepared by placing 50 g of rice into 500-mL glass bottles, adding 65 mL of sterile distilled water, soaking overnight, and sterilizing by autoclaving at 121 °C and 1 atm for 15 min. Growing fungal cultures were inoculated onto the rice medium and incubated at room temperature for 7 days (observing growth); for extended secondary-metabolite induction, the bottles may be incubated up to 4 weeks as required for macroscopic development (Handayani et al., 2022).

Extraction of secondary metabolites

After incubation, fungal cultures on rice were extracted with ethyl acetate. Cultured rice (including mycelium) was macerated and mixed with ethyl acetate. The extraction was repeated three times. The combined ethyl acetate extracts were filtered and concentrated under reduced pressure using a rotary evaporator at 40 °C to yield a viscous

crude extract. Extracts were weighed and stored at 4°C until bioassay (Handayani et al., 2022).

Antibacterial screening Kirby–Bauer disc diffusion

The antibacterial activity of crude ethyl acetate extracts was evaluated using the Kirby–Bauer disc diffusion method. Test strains (*Staphylococcus aureus* ATCC 25922 and *Escherichia coli* ATCC 29213) were reactivated by streaking onto Nutrient Agar slants and incubated at 37 °C for 24 h. Bacterial suspensions were prepared to match the 0.5 McFarland standard (approximately 1.5×10^8 CFU mL^{-1}). Sterile agar plates were then inoculated by evenly swabbing the bacterial suspension over the surface. Sterile paper discs (6 mm) were impregnated with the fungal extracts (10% w/v) and placed on the inoculated plates. Tetracycline discs served as positive controls, while solvent-only discs were used as negative controls. All plates were incubated at 37 °C for 18–24 h, and the diameter of the inhibition zones was measured in millimetres (Handayani et al., 2019).

Thin-layer chromatography (TLC) and phytochemical screening

The ethyl acetate extract of isolate PE2 was analyzed by thin-layer chromatography (TLC) to assess its secondary metabolite profile. TLC was performed on silica gel 60 F254 plates using n-hexane: ethyl acetate: dichloromethane (1:2:3, v/v/v) as the mobile phase. After development, the plates were air-dried and visualized under UV light at 254 and 366 nm. For further phytochemical screening, the developed plates were sprayed with specific detecting reagents: Dragendorff's reagent (alkaloids), ferric chloride reagent (phenolics), and Liebermann–Burchard reagent (steroids and triterpenoids). The appearance of color was recorded as a positive detection.

RESULT AND DISCUSSION

Macroscopic morphological characteristics of endophytic fungi

A total of five endophytic fungal isolates were successfully obtained from *Pachyrhizus erosus* (L.) Urb. (Bengkuang), as shown in Figure 1. Each isolate exhibited distinct macroscopic characteristics when cultured on Sabouraud Dextrose Agar (SDA). Isolates PE1 and PE3, both obtained from the tuber, formed filamentous colonies; PE1 was dark green with concentric rings characterized by a white margin at the colony edge, while PE3 was light green with similar concentric growth patterns. Isolates PE2 and PE4, also derived from the tuber, displayed contrasting appearances: PE2 produced cottony colonies that were predominantly white with a yellowish-brown centre and lacked

concentric rings, whereas PE4 developed dark brown powdery colonies, also without concentric growth. Finally, isolate PE5, obtained from the leaf, formed cream-white cottony colonies without concentric rings.

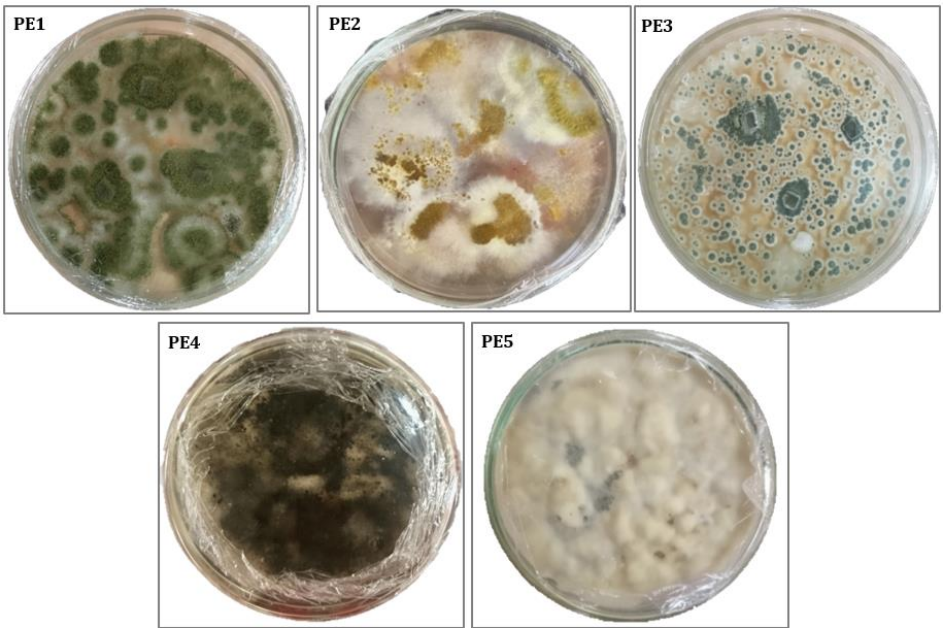

Figure 1. Macroscopic morphological characteristics of endophytic fungi (PE1–PE5) isolated from *Pachyrhizus erosus* (L.) Urb.

Antibacterial screening result

All fungal extracts exhibited inhibitory effects against *S. Aureus* and *E. coli*, although with varying potencies, as shown in Figure 2.

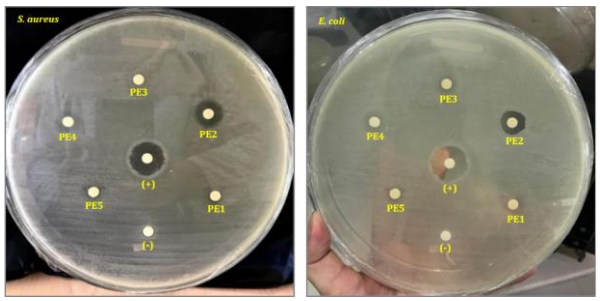

Figure 2. Antibacterial activity of ethyl acetate extracts of endophytic fungi (PE1–PE5) from *Pachyrhizus erosus* against *S. aureus* ATCC 29213 (left) *and E. coli* ATCC 25922 (right)

Among the isolates, PE2 exhibited the strongest antibacterial activity against both *S. aureus* and *E. coli*, with inhibition zones closest to the positive control, suggesting its high potential as a source of active metabolites. Moderate inhibition was observed for PE4, while PE1, PE3, and PE5 showed weaker activity. These findings also indicate that

tuber-derived fungi (PE1–PE4) generally displayed higher antibacterial effects than the leaf-derived isolate (PE5). As expected, the positive control (tetracycline) produced the largest inhibition zones, validating the assay (Table 1). Compared with plant extracts, our fungal isolate PE2 showed stronger antibacterial activity against *S. aureus* and *E. coli*; for context, previous studies on *P. erosus* tuber/seed extracts reported inhibition zones against *S. aureus* ranging from 6.0 mm (low concentrations) up to 13.3 mm (Nurfitriani et al., 2021).

Table 1. Antibacterial activity of ethyl acetate extracts of endophytic fungi isolated from *Pachyrhizus erosus* (tuber and leaf)

Bacterial strain	Sample (concentration 10% b/v)	Inhibition zone (mm, Mean ± SD)
S. aureus ATCC 29213	PE1	8.0 ± 1.13
	PE2	15.7 ± 1.40
	PE3	7.2 ± 2.30
	PE4	11.7 ± 1.37
	PE5	9.5 ± 0.82
	Positive control	18.0 ± 1.20
E. coli ATCC 25922	PE1	7.6 ± 3.15
	PE2	14.4 ± 0.32
	PE3	7.6 ± 3.35
	PE4	11.5 ± 1.16
	PE5	9.3 ± 1.67
	Positive control	17.9 ± 1.40

Endophytic fungi isolated from different plant tissues often exhibit variation in their metabolite profiles and biological activities (Nazir et al., 2024). They are increasingly regarded as promising sources of novel antimicrobial compounds with relevance against both Gram-positive and Gram-negative bacteria (Bansal et al., 2025). In the present work, PE2 showed the highest antibacterial activity, although its inhibition zones were still lower than those of tetracycline, which is consistent with earlier reports. Comparable results were documented in *Chrysanthemum indicum*, where ethyl acetate extracts of fungal endophytes suppressed bacterial growth but did not reach the potency of standard antibiotics (Ratte et al., 2023). Likewise, endophytic fungi associated with mangroves demonstrated measurable activity against *S. aureus* and *E. coli*, further supporting the idea that endophytes serve as valuable reservoirs of antibacterial metabolites (Mendieta-Brito et al., 2024). Such bioactivities are typically attributed to chemically diverse metabolites, including polyketides, nonribosomal peptides, terpenes,

phenolics, and alkaloids, which are biosynthesized through Polyketide Synthase (PKS) and Nonribosomal Peptide Synthetase (NRPS) pathways (Nazir et al., 2024). The pronounced activity of PE2, therefore, suggests that this isolate may produce a particularly effective set of secondary metabolites.

TLC Profiling and Secondary Metabolite Group Identification of Isolate PE2

The ethyl acetate extract of isolate PE2 was subjected to TLC analysis to examine its chemical profile. Visualization under UV, as shown in Figure 3, and with spraying reagents revealed multiple spots, indicating that PE2 produces a diverse range of secondary metabolites (Table 2).

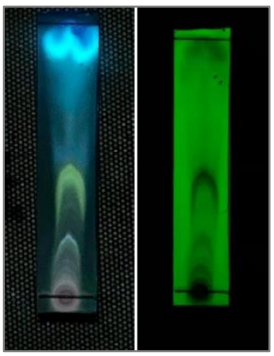

Figure 3. TLC profile of PE2 extract (eluent: n-hexane: ethyl acetate: dichloromethane, 1:2:3), observed under UV 254 (right) and 366 nm (left)

Table 2. Chemical profiling of PE2 extract by TLC with detecting reagents

TLC visualization	Reagent	Interpretation
	Dragendorff's for alkaloid detection	Negative
	$FeCl_3$ for fenolics detection	Positive
	Liebermann–Burchard for Steroids / Triterpenoids detection	Positive

The PE2 extract reacted positively with the Liebermann–Burchard reagent, supporting the presence of lipophilic terpenoid-type constituents. This finding aligns with

phytochemical reports on *Pachyrhizus erosus*, which contains flavonoids, triterpenoids, and rotenoids—compound classes widely associated with antibacterial activity (Jaiswal et al., 2021). Taken together, these observations suggest that tuber-associated endophytic fungi such as PE2 may either biosynthesize metabolites structurally related to their host plant or biotransform plant-derived precursors into more potent antibacterial derivatives

CONCLUSION

Endophytic fungi isolated from *Pachyrhizus erosus* demonstrated notable antibacterial potential, particularly isolate PE2, which exhibited strong inhibition against both *Staphylococcus aureus* and *Escherichia coli*. TLC profiling confirmed the presence of phenolic and triterpenoid-type metabolites, consistent with the phytochemical composition of the host plant. Taken together, the role of tuber-associated endophytes as promising sources of bioactive compounds supports their potential application in the discovery of alternative antibacterial agents.

ACKNOWLEDGE

The authors would like to express their sincere gratitude to:

1. All co-authors for their valuable contributions to this study.
2. Affiliated institutions for providing facilities and support during the research.

REFERENCES

Bansal, S., Joshi, M., Mahajan, P., Sharma, S., Kaur, S., Thakur, B., ... & Tripathi, M. (2025). Endophytic fungi as a source of antimicrobial compounds against emerging methicillin-resistant *Staphylococcus aureus*. *Mycology*, 1–28.

Handayani, D., Dwinatrana, K., & Rustini, R. (2022). Antibacterial compound from marine sponge derived fungus *Aspergillus sydowii* DC08. *Rasayan Journal of Chemistry, 15*(4), 2485–2492.

Handayani, D., Ruslan, R., Nestianda, O., Fajrina, A., & Tallei, T. E. (2019). Cytotoxic and antimicrobial activities of ethyl acetate extract of mangrove plant *Scyphiphora hydrophyllacea* CF Gaertn—Associated fungi. *Journal of Applied Pharmaceutical Science, 9*(6), 075–079.

Jaiswal, V., Chauhan, S., & Lee, H. J. (2021). The bioactivity and phytochemicals of *Pachyrhizus erosus* (L.) Urb.: A multifunctional underutilized crop plant. *Antioxidants, 11*(1), 58.

Kjer, J., Debbab, A., Aly, A. H., & Proksch, P. (2010). Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. *Nature Protocols, 5*(3), 479–490.

Mendieta-Brito, S., Sayed, M., Son, E., Kim, D. S., Dávila, M., & Pyo, S. H. (2024). Identification, characterization, and antibacterial evaluation of five endophytic fungi from *Psychotria poeppigiana* Müll. Arg., an Amazon plant. *Microorganisms, 12*(8), 1590.

Nazir, A., Puthuveettil, A. R., Hussain, F. H. N., Hamed, K. E., & Munawar, N. (2024). Endophytic fungi: Nature's solution for antimicrobial resistance and sustainable agriculture. *Frontiers in Microbiology, 15,* 1461504.

Nurfitriani, A., Mahendradatta, M., & Laga, A. (2021, August). Antibacterial effectiveness of purple yam (*Dioscorea alata* L) sap extract in inhibiting the growth of *Staphylococcus aureus* & *Escherichia coli*. In *10th International Seminar and 12th Congress of Indonesian Society for Microbiology (ISISM 2019)* (pp. 5–11). Atlantis Press.

Ortega, H. E., Torres-Mendoza, D., & Cubilla-Rios, L. (2025). Antibacterial compounds isolated from endophytic fungi reported from 2021 to 2024. *Antibiotics, 14*(7), 644.

Rahminiwati, M., Batubara, I., Nurcholis, W., Safinatunajah, R. R., Sari, F. E., & Sulistiyono, F. D. (2025). Antibacterial and antibiofilm properties of jicama (*Pachyrhizus erosus*) seed extract against *Streptococcus mutans*: Identification of key bioactive compounds using bioautography and LC-MS/MS analysis. *Tropical Journal of Natural Product Research, 9*(1).

Ratte, T. A., Fatimawali, F., Tallei, T. E., Suoth, E. J., Antasionasti, I., & Yamlean, P. (2023). Evaluation of antibacterial properties from endophytic fungi of *Chrysanthemum indicum* (L.) flowers against *Escherichia coli* and *Staphylococcus aureus*. *Grimsa Journal of Science Engineering and Technology, 1*(2), 71–77.

Rosdee, S., Wisessombat, S., Tayeh, M., Malakul, R., Phanaksri, T., & Sianglum, W. (2025). Antibacterial activity of the endophytic fungal extracts and synergistic effects of combinations of ethylenediaminetetraacetic acid (EDTA) against *Pseudomonas aeruginosa* and *Escherichia coli*. *PeerJ, 13,* e19074.

Sandrawati, N., Ningsih, W., Layla, R., Putra, A. E., Ismed, F., Tallei, T. E., & Handayani, D. (2023). Endophytic fungi from mangrove plant *Acanthus ilicifolius* L.: Antimicrobial, anticancer, and species determination. *Trends in Sciences, 20*(7), 5089–5089.

World Health Organization (WHO). (2024). *Antimicrobial resistance fact sheet*. World Health Organization. https://www.who.int/news-room/factsheets/detail/antimicrobial-resistance

Xiang, J., Huang, S., Wu, X., He, Y., Shen, H., Tang, S., ... & Luo, Y. (2025). Phytochemical profile and antioxidant activity of the tuber and peel of *Pachyrhizus erosus*. *Antioxidants, 14*(4), 416.