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ARTIKEL

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF ENDOPHYTIC FUNGI EXTRACTS FROM THE FLOWER OF PURUN TIKUS (*Eleocharis dulcis* (Burm.f.) Trin. Ex Hensch.)

[*Aktivitas Antioksidan dan Antibakteri Ekstrak Jamur Endofit dari Bunga Purun Tikus (Eleocharis dulcis* (Burm.f.) Trin. Ex Hensch.)]

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ABSTRACT

Purun Tikus (*Eleocharis dulcis* (Burm.f.) Trin. Ex Hensch.) is a wetland plant native to Southeast Asia known to contain various bioactive secondary metabolites. However, studies on endophytic fungi associated with the flowers of this plant remain limited, even though endophytes are often capable of producing compounds similar to or even more potent than those of their host plants. This study aimed to identify and evaluate the antioxidant and antibacterial activities of endophytic fungi isolated from the flowers of *E. dulcis*. Isolation was carried out using surface sterilization and cultivation on Potato Dextrose Agar (PDA), followed by identification based on macroscopic and microscopic characteristics. Three isolates were successfully obtained: EDF1 (*Neopestalotiopsis* sp.), EDF2 (*Lasiodiplodia theobromae*), and EDF3 (*Rhizopus* sp.). Secondary metabolites were extracted using ethyl acetate, while antioxidant and antibacterial activities were evaluated using the DPPH method and agar disc diffusion method, respectively, against *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. The results showed that each isolate exhibited different levels of bioactivity. Among them, EDF2 (*L. theobromae*) demonstrated the highest activity, with an IC_{50} value of 19.149 µg/mL (categorized as very strong) and antibacterial inhibition exceeding 80% against all tested bacteria. These findings indicate that *L. theobromae* has strong potential as a natural source of bioactive compounds that can be further developed as candidates for phytopharmaceuticals, antimicrobial agents, and natural antioxidants through subsequent in vivo studies.

Keywords: *Eleocharis dulcis*, Endophytic Fungi, *Lasiodiplodia theobromae*, Antioxidant, Antibacterial

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ABSTRAK

Purun Tikus (Eleocharis dulcis) merupakan tanaman rawa Asia Tenggara yang diketahui mengandung berbagai metabolit sekunder bioaktif. Namun, kajian mengenai jamur endofit pada bagian bunga tanaman ini masih terbatas, padahal jamur endofit sering menghasilkan senyawa yang serupa atau bahkan lebih kuat dibandingkan senyawa tanaman inangnya. Penelitian ini bertujuan untuk mengidentifikasi serta mengevaluasi aktivitas antioksidan dan antibakteri jamur endofit yang diisolasi dari bunga E. dulcis. Isolasi dilakukan menggunakan metode sterilisasi permukaan dan penanaman pada media Potato Dextrose Agar (PDA), kemudian diidentifikasi berdasarkan karakter makroskopis dan mikroskopis. Tiga isolat berhasil diperoleh, yaitu EDF1 (Neopestalotiopsis sp.), EDF2 (Lasiodiplodia theobromae), dan EDF3 (Rhizopus sp.). Ekstraksi metabolit sekunder dilakukan dengan pelarut etil asetat, sedangkan uji aktivitas dilakukan menggunakan metode DPPH untuk antioksidan dan metode difusi cakram untuk antibakteri terhadap Salmonella typhi, Escherichia coli, Staphylococcus aureus, dan Bacillus subtilis. Hasil menunjukkan bahwa setiap isolat memiliki bioaktivitas berbeda. Isolat EDF2 (L. theobromae) memiliki aktivitas antioksidan paling tinggi dengan nilai IC_{50} sebesar 19,149 µg/mL (kategori sangat kuat) dan daya hambat antibakteri lebih dari 80% terhadap semua bakteri uji. Dengan demikian, L. theobromae berpotensi sebagai sumber alami senyawa bioaktif yang dapat dikembangkan sebagai kandidat fitofarmaka, agen antimikroba, dan antioksidan alami melalui uji lanjutan in vivo.

Kata Kunci: *Eleocharis dulcis*, *Jamur Endofit, Lasiodiplodia theobromae*, *Antioksidan, Antibakteri*

INTRODUCTION

Excessive production of free radicals in the human body can cause oxidative stress, which plays a key role in the development of various degenerative diseases such as cancer, diabetes, and neurodegenerative disorders (Chandimali *et al*., 2025; Goyal *et al*., 2024). Antioxidants function to neutralize free radicals and protect cells from oxidative damage (Pizzino *et al*., 2017). However, the use of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) has raised health concerns due to their potential toxic and carcinogenic effects (Felter *et al*., 2021). Therefore, exploring natural antioxidant sources from microorganisms and plants has become an important focus in biomedical research (Mallik *et al*., 2024; Pooja *et al*., 2025).

On the other hand, antibiotic resistance continues to increase as a result of excessive and inappropriate antibiotic use. Resistant pathogens such as *Staphylococcus aureus* and *Escherichia coli* pose a serious global health threat (Bhuiyan *et al*., 2025). Hence, there is an urgent need to discover new antibacterial agents from alternative, safer, and more effective biological sources (Hnamte *et al*., 2024). Endophytic microorganisms have attracted considerable attention due to their ability to produce unique bioactive metabolites, including compounds with antioxidant and antibacterial activities (Caruso *et al*., 2022; Hashem *et al*., 2023).

Endophytic fungi, which reside within plant tissues without causing disease symptoms, have been widely reported to produce bioactive secondary metabolites with significant pharmacological potential (Gurgel *et al*., 2023; Selim *et al*., 2025). Their ability to mimic or modify host-derived compounds makes them valuable sources for the discovery of novel natural products (Enyi *et al*., 2025). Compared to their host plants, *endophytic fungi* offer faster cultivation, scalable fermentation, and sustainable production of bioactive compounds. They can also produce metabolites with stronger or unique biological activities, making them promising sources for pharmaceutical and biotechnological applications (El-Nagar *et al*., 2024).

Purun Tikus (*Eleocharis dulcis*), a characteristic wetland plant native to Southeast Asia, exhibits strong ecological adaptability and contains diverse secondary metabolites (Nurnawati *et al*., 2019). However, studies on endophytic fungi associated with the flowers of *E. dulcis* remain scarce, despite the possibility that this plant part may harbor microorganisms capable of producing valuable bioactive compounds (You *et al*., 2015). The choice of *E. dulcis* as a source of endophytic fungi is further justified by its adaptation to challenging wetland and acidic soil conditions, as such stress tolerance often correlates with the production of novel or potent secondary metabolites (Amalia *et al*., 2023). Additionally, various parts of the plant have demonstrated pharmacological activities including antioxidant, antibacterial, anti-inflammatory and hypoglycaemic effects (Gu *et* *al*., 2021; Yang *et al*., 2020), which implies that its internal microbial symbionts may inherit or modify similar biosynthetic capabilities.

Based on these considerations, this study was conducted to explore the antioxidant and antibacterial activities of endophytic fungal extracts isolated from the flowers of *E. dulcis*, providing new insights into potential natural sources of bioactive compounds with prospective applications in the field of health sciences.

MATERIALS AND METHODS

Sample Preparation

The research samples consisted of fresh *E. dulcis* (purun tikus) plants collected from wetlands in Palembang City, South Sumatra Province, Indonesia (GPS coordinates -3.010118º S, 104.771604º E). The samples were characterized as healthy, mature plants with heights ranging from 0.5 to 2 meters, glossy dark green stems, and fully developed flowers at the stem tips. The plant material was identified at Yayasan Generasi Biologi Indonesia, under Service Order ID BT-072478, ensuring accurate taxonomic verification and traceability of the material used in this study.

Table 1. Information about sample

Name of Sample *(Nama Sampel)*	**Latitude** *(Lintang)*	**Longitude** *(Bujur)*	**Total Dissolved Solid** *(TDS)* **(ppm)**	**pH** *(pH)*	**Temperature** *(Suhu)* **(ºC)**	**Sampel Colector** *(Kolektor Sampel)*	**Date** *(Tanggal)*	**Time**
Eleocharis dulcis (Burm.f.) Trin. ex Hensch.	-3.010118º	104.771604º	404	2,62	33,6	Kurratul ʻAini	05 August 2025	05.04

Isolation of Endophytic Fungi

The flower samples of *E. dulcis* were first washed under running water for approximately 5 minutes to remove surface debris. Surface sterilization was then performed: each sample was wiped with 70% ethanol for about 3 minutes, followed by rinsing with sterile distilled water for 1 minute. Plant tissues were then cut as required and immersed in natrium hypochlorite (NaOCl) for approximately 30 seconds, followed by a second rinse in 70% ethanol for 30 seconds and a final rinse with sterile distilled water for 1 minute. The sterilized plant tissues were aseptically cut into approximately 1 × 0.5 cm. Each segment was sampled in triplicate, with three independent plants used as biological replicates, and each segment considered a technical replicate for subsequent endophytic fungal isolation. (Habisukan, 2021).

The prepared segments were inoculated onto potato dextrose agar (PDA) plates and incubated at room temperature for 3–7 days. Fungal colonies that emerged on the PDA medium were subsequently purified by transferring them to fresh PDA plates, followed by incubation at room temperature for 48 hours (Aini, *et al*., 2025; Oktiansyah *et al*., 2023)

Morphological Characterization for the Identification of Endophytic Fungi

Endophytic fungi were identified based on their phenotypic characteristics, including both macroscopic and microscopic features. Macroscopic observations of the colonies included surface and reverse coloration, colony texture (e.g., cottony, granular, powdery, or slimy), presence of exudate droplets, radial lines, and concentric rings. Microscopic examination was carried out by inoculating fungal samples and preparing slides using the Henrici slide culture technique. The morphology was observed under a Hirox digital microscope, with attention to spore morphology and hyphal septation. Fungal identification was based on a combination of macroscopic and microscopic traits, compared against standard fungal identification references (Walsh *et al*., 2018; Watanabe, 2010). For long-term preservation and future studies, pure fungal cultures were maintained on potato dextrose agar (PDA) slants at 4 °C and periodically subcultured every 3–6 months. Additionally, selected isolates were preserved in 20% glycerol stocks at –80 °C to ensure genetic stability and viability over time (Saxena & Gupta, 2019).

Extraction of *E. dulcis* Flowers

Fresh *E. dulcis* flowers were sun-dried in the shade and then ground into a fine powder. Extraction was carried out using the maceration method with methanol solvent at a ratio of flower powder: solvent of 1:10 (w/v), for 24 hours at room temperature with mild agitation, then the filtrate was concentrated using a rotary evaporator until a thick extract was obtained. After obtaining the crude extract, it was stored at -20°C until used for bioactive activity testin (Baehaki *et al*., 2021).

Extraction and Cultivation

Pure endophytic fungal isolates were cultivated in Potato dextrose broth (PDB). Fungal colonies were sampled using a 5 mm diameter cork borer, with 20 agar plugs inoculated into 300 mL of PDB. The cultures were then incubated statically at room temperature for 30 days. After incubation, the mycelium was separated from the culture broth using filter paper. Ethyl acetate was added to the culture medium at a 1:1 ratio, and liquid-liquid extraction was performed three times. The ethyl acetate extract was separated from the aqueous phase and concentrated using a rotary evaporator to obtain a crude extract (Habisukan., 2022; Hapida., 2021).

Antioxidant Activity Test

The antioxidant activity was evaluated by mixing 0.2 mL of the sample extract in ethanol at various concentrations (1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL) with 3.8 mL of 0.05 mM DPPH solution. The mixture was homogenized and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer, and the antioxidant activity was expressed as the percentage of DPPH radical inhibition. Ascorbic acid prepared at the same concentrations (1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL) as the test samples served as a positive control for comparison of antioxidant capacity (Molyneux P, 2003). Plotting the percentage of inhibition against concentration series allowed for the expression of the antioxidant activity in IC_{50} values.

Antibacterial Activity Test

The antibacterial activity of the extract was evaluated using the agar disc diffusion method against four bacterial strains: *Salmonella typhi* (IPCCCB.11.669), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Bacillus subtilis* (ATCC 6633). Bacterial suspensions were standardized to 1.5×10^8 CFU/mL, corresponding to 0.5 McFarland. A total of 100 µL of each bacterial suspension was inoculated onto Mueller-Hinton agar (MHA) plates containing 20 mL of medium. Sterile discs (6 mm diameter) impregnated with 20 µL of the concentrated extract in 10% DMSO were placed on the inoculated agar. Tetracycline (20 µL/disc) served as the positive control. Plates were incubated at 37 °C, and the resulting inhibition zones were measured. Antibacterial activity of the extract was expressed as a percentage relative to the standard antibiotic and categorized as strong (≥70% inhibition), moderate (50–70%), or weak (<50%) (Budiono *et al*., 2019).

RESULTS

A total of three endophytic fungal isolates were successfully obtained from the flowers, designated as EDF1, EDF2 and EDF3. The macroscopic characteristics of the colonies exhibited diversity in shape and color, while the microscopic features displayed distinctive traits specific to each isolate (Figure 1). The colony colors of the endophytic fungi were gray, cream, and black. The detailed macroscopic and microscopic characteristics of the three endophytic fungal isolates obtained from the flowers are presented in Tables 2 and 3.

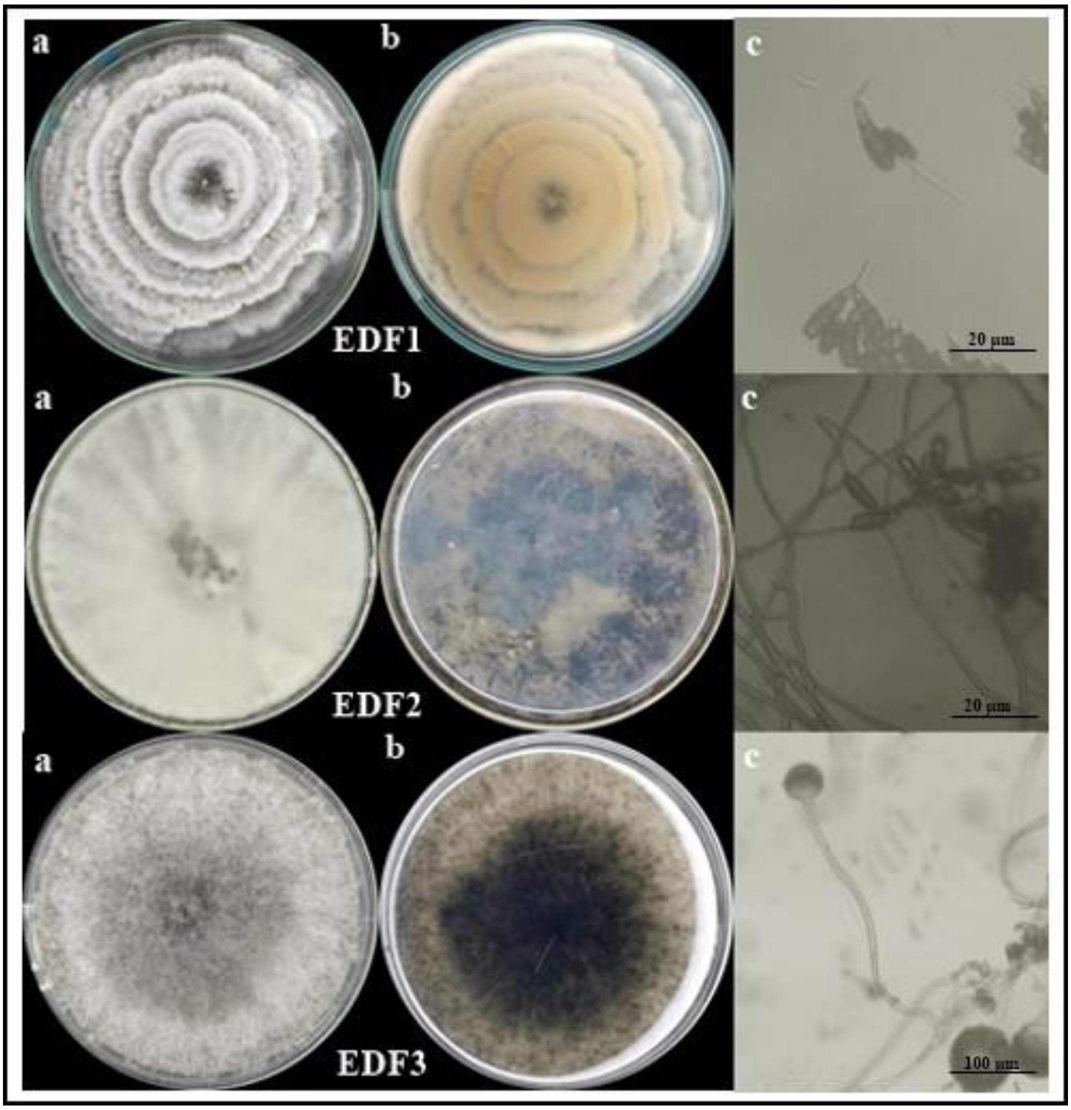

Figure 1. Morphological characteristics of endophytic fungi isolated from the flowers of *E. dulcis* on PDA medium. Notes: (a) front view, (b) reverse view, (c) microscopic characteristics (1000× magnification). (*Karakteristik morfologi jamur endofit yang diisolasi dari bunga E. dulcis pada media PDA. Catatan: tampak depan, b: tampak belakang, c: karakteristik mikroskopis (1000x*).

Table 2. Macroscopic Characteristics of Endophytic Fungi from the Flowers of *E. dulcis (Karakteristik Makroskopis Jamur Endofitik dari Tumbuhan Bunga E. dulcis*).

Code (*Code*)	**Surface Colony** (*Warna Permukaan koloni*)	**Reverse Colony** (*Warna Sebalik Koloni*)	**Structure** (*Struktur*)	**Elevation** (*Bentuk Puncak*)	**Pattern** (*Pola*)	**Exudate Drops** (*Tetes Eksudat*)	**Radial line** (*Garis Radial*)	**Concentric circle** (*Lingkar Konsetris*)
EDF1	Grayish White (*Putih Keabuan*)	Yellowish Brown (*Kuning Kecoklatan*)	Floccose (*Berbulu*)	Umbonate (*Timbul tengah*)	Zonate (*Membentuk Zonasi*)	-	√	√
EDF2	Grayis White (*Putih Keabuan*)	Grayish Black (*Hitam Keabuan*)	Cottonty (*Kapas*)	Flat (*Datar*)	Irregular (*Tidak beraturan*)	-	-	-
EDF3	Gray (*Abu-Abu*)	Blackish Brown (*Hitam Kecoklatan*)	Velvety (*Beludru*)	Raised (*Menunggi*)	Circular (*Membentuk Sirkular*)	-	-	-

Table 3. Microscopic Characteristics of Endophytic Fungi from the Flowers of *E. dulcis* (*Karakteristik Makroskopis Jamur Endofitik dari Tumbuhan Bunga E. dulcis*).

Isolate (*Isolat*)	**Spore** (*Spora*)	**Shape** (*Bentuk*)	**Hyphae** (*Hifa*)	**Distinctive Characteristic** (*Karakteristik Khas*)	**Species of Identification** (*Spesies Identifikasi*)
EDF1	Conidia (*Konidia*)	Ellipsoidal	Septat	Short-chain conidia, pale in color (*Konidia berantai pendek, warna pucat*)	*Neopestalotiopsis* sp.
EDF2	Conidia (*Konidia*)	Ellipsoidal	Septat	Dark brown conidia, arranged in clusters (*Konidia berwarna coklat kehitaman, bersusun*)	*Lasiodiplodia theobromae*
EDF3	Sporangiospora	Globose (*Bulat*)	Non-Septat	Spherical sporangium, aseptate hyphae (*Sporangium bulat, hifa tidak bersekat*)	*Rhizopus* sp.

The identification of endophytic fungi was carried out based on both macroscopic and microscopic characteristics following standard mycological identification manuals (Walsh *et al*., 2018; Watanabe, 2010). Macroscopic characteristics such as colony color (surface and reverse), texture, elevation, and growth pattern were compared with morphological keys for common endophytic genera. Microscopic characteristics were then observed using the Henrici slide culture technique, focusing on spore morphology (shape, color, septation) and hyphal structures (presence of septa, branching, or special reproductive structures). Identification proceeded in a stepwise narrowing process, starting from genus, level grouping based on macroscopic features, followed by confirmation at the species level using distinctive microscopic traits compared to taxonomic descriptions from relevant keys and databases.

Extracts from endophytic fungi isolated from *E. dulcis* flowers demonstrated both antibacterial and antioxidant activities (Table 4).

Table 4. Percentage of antibacterial activity of endophytic fungal extract compared to that of tetracycline and antioxidant activity compared to that of ascorbic acid as a standard (*Persentase aktivitas antibakteri ekstrak jamur endofit dibandingkan dengan tetrasiklin dan aktivitas antioksidan dibandingkan dengan asam askorbat sebagai standar*).

Code Isolate *(Kode Isolate)*	**Sample** *(Sampel)*	**Weight of Extract** *(Berat Ekstrak)* **(g)**	**IC_{50} (µg/mL)**	**Percentage of antibacterial activity (%)** *(Presentase Aktivitas Antibakteri)*			
				E. coli	***S. typhi***	***S. aureus***	***B. subtilis***
	E. dulcis Flower Extract (*Ekstrak Bunga E. dulcis*)	2,1	26,18***	60,12±0,94**	61,4±1,18**	57,1±0,90**	52,4±0,15**
EDF1	*Neopestalotiopsis* sp.	2,4	29,96***	79,8±0,14***	80,1±0,04***	74,5±1,14***	71,4±0,12***
EDF2	*Lasiodiplodia theobromae*	3,0	19,15****	81,7±1,25***	80,9±0,12***	82,8±0,05***	85,0±0,33***
EDF3	*Rhizopus* sp.	2,1	78,39***	63,2±1,39**	60,9±0,03**	66,1±0,16**	69,4±0,67**
Positive Control (*Kontrol Positif*)		Ascorbic Acid (*Asam Askorbat*) 10,08****		Tetracycline (*Tetrasiklin*) 100***	Tetracycline 100***	Tetracycline 100***	Tetracycline 100***

Note: Antioxidant activity IC_{50} (µg/mL): ****very strong < 20 µg/mL ***strong < 100 µg/mL; **moderat 100-500 µg/mL; * weak > 500 µg/mL. Antibacterial activity percentage: *** strong (≥ 70%), **moderate (50-70%), and *weak (< 50%). (Catatan: Aktivitas antioksidan IC_{50} (µg/mL): ****sangat kuat < 20 µg/mL ***kuat < 100 µg/mL; **sedang 100-500 µg/mL; *lemah > 500 µg/mL.Persentase aktivitas antibakteri: *** kuat (≥ 70%), **sedang (50-70%), dan *lemah (< 50%).)

DISCUSSION

Based on morphological identification, the isolated endophytic fungi obtained from *E. dulcis* flowers were identified as follows: EDF1 as *Neopestalotiopsis* sp., EDF2 as *Lasiodiplodia theobromae*, and EDF3 as *Rhizopus* sp. These results are consistent with previous reports indicating that these three fungal genera are commonly associated with tropical plants, either as endophytes or as pathogens infecting flowers, fruits, and vegetative organs (Mendieta-brito *et al*., 2024). Several studies have demonstrated that plant pathogenic fungi can also exist as endophytes in medicinal plants without causing visible disease symptoms and often exhibit high bioactivity due to the production of secondary metabolites structurally similar to those found in their host plants. The ability of endophytic fungi to mimic or complement the metabolic pathways of their host plants further supports their relevance in research (Akram *et al*., 2023).

Endophytic fungi are known to produce a wide range of secondary metabolites through their invasive and adaptive capabilities. Fungi isolated from medicinal plants have been proven to exhibit high bioactivity in various studies, suggesting that *Neopestalotiopsis* sp., *Lasiodiplodia theobromae*, and *Rhizopus* sp. associated with *E. dulcis* potentially synthesize secondary metabolites analogous to those of their host plant (Gupta *et al*., 2023). Previous research has also reported that these genera, when isolated from medicinal plants, possess strong biological activity attributed to the presence of metabolites resembling host-derived compounds (Doifode *et al*., 2023; Gupta *et al*., 2023).

Table 4 presents the bioactivity results of the endophytic fungal extracts isolated from *E. dulcis* flowers and their host plant. Based on the data, each isolate exhibited varying degrees of biological activity against the tested bacterial strains and in terms of antioxidant potential. Among all isolates tested, EDF2 demonstrated the most prominent bioactivity, characterized by a very strong antioxidant capacity and high antibacterial inhibition against all four tested bacteria.

The EDF2 isolate, identified as *Lasiodiplodia theobromae*, showed an IC_{50} value of 19.149 µg/mL, which falls within the very strong category (IC_{50} < 20 µg/mL). This finding indicates that the secondary metabolites produced by EDF2 possess a high capacity for radical scavenging and oxidative stabilization. This result aligns with the findings of Elfita *et al*., (2023),who reported that the ethyl acetate extract of *L. theobromae* isolated from *Peronema canescens* leaves exhibited very strong antioxidant activity (IC_{50} = 12.2 µg/mL), comparable to that of ascorbic acid (IC_{50} = 10.1 µg/mL). The antioxidant potential of *L. theobromae* is closely associated with the presence of phenolic and hydroxyl functional groups in its secondary metabolites, which play a crucial role in stabilizing free radicals through electron donation mechanisms.

In addition to its antioxidant potential, EDF2 exhibited strong antibacterial activity against *E. coli*, *S. typhi*, *S. aureus*, and *B. subtilis*, with inhibition percentages exceeding 80%. These results indicate that *L. theobromae* possesses broad-spectrum antibacterial properties, acting effectively against both Gram-positive and Gram-negative bacteria. This observation is consistent with Elfita *et al*., (2023), who reported that the ethyl acetate extract of *L. theobromae* exhibited MIC values ranging from 32 to 128 µg/mL, showing strong inhibitory effects particularly against *S. typhi* and *B. subtilis*.

Interestingly, the high biological activity of EDF2 as *L. theobromae* not only reflects its known pathogenic potential in certain tropical plants but also highlights its adaptive capability as a mutualistic endophyte. Within this symbiotic relationship, *L. theobromae* may synthesize secondary metabolites that enhance host defense mechanisms against biotic and abiotic stress while simultaneously producing bioactive compounds with pharmaceutical value (Adeleke & Babalola, 2021).

In addition to assessing the bioactive properties of the endophytic fungal isolates, this study also evaluated the antioxidant and antibacterial activities of the *E. dulcis* flower extract. The results showed that the flower extract exhibited strong antioxidant activity (IC_{50} = 26.183 µg/mL), although it was not as potent as the endophytic isolates, particularly EDF2. In the antibacterial assay, the flower extract also demonstrated moderate activity, with inhibition percentages ranging from 52–61% against *E. coli*, *S. typhi*, *S. aureus*, and *B. subtilis*. Although this activity was lower compared to the endophytic isolates (especially EDF1 and EDF2), the findings still indicate that the *E. dulcis* flower itself has potential as a natural antibacterial agent. According to Wen *et al*., (2022) and Zakariyah *et al*., (2024) the relatively low antibacterial activity may be attributed to the lower concentration of active metabolites or the complex nature of the extract, which may result in less concentrated bioactive compounds compared to endophytic isolates that produce specific metabolites.

Interestingly, the activity of the flower extract was consistently lower than that of all endophytic fungal isolates. This finding is in line with Hashem *et al*., (2023) how reported that endophytic microorganisms often produce metabolites with higher potency than their host tissues. This phenomenon is thought to occur because endophytes possess specialized biosynthetic pathways that enable the production of tertiary bioactive compounds and phenolic derivatives in higher concentrations than those found in the corresponding plant tissues (Slama *et al*., 2021).

The dual activity exhibited by EDF2 namely, its very strong antioxidant capacity and broad-spectrum antibacterial effect indicates that *L. theobromae* is a promising source of bioactive secondary metabolites. These compounds hold significant potential for further development as candidate materials for phytopharmaceuticals, antimicrobial agents, and natural antioxidant compounds.

CONCLUSION

This study revealed the diversity of endophytic fungi isolated from *E. dulcis* flowers, including *Neopestalotiopsis* sp., *Lasiodiplodia theobromae*, and *Rhizopus* sp. Among these isolates, *L. theobromae* demonstrated the most prominent biological activity, characterized by very strong antioxidant potential (IC_{50} = 19.149 µg/mL) and high antibacterial activity against both Gram-positive and Gram-negative bacteria. The biological potency of EDF2 suggests that *L. theobromae* associated with *E. dulcis* may serve as a valuable source of bioactive metabolites. These findings highlight its potential for further exploration in pharmaceutical or natural product applications and provide a preliminary foundation for subsequent in-depth studies aimed at discovering novel antioxidants and antimicrobial compounds. These findings provide a scientific basis for further development of *L. theobromae* from *E. dulcis* as a potential source of bioactive compounds, which should be validated through further in vivo studies and molecular confirmation (ITS rDNA sequencing).

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AUTHOR CONTRIBUTIONS

K.A. designed the research and supervised all the processes. S. collected and analyzed the data. S.E.B., S.W.F and E. assisted with the laboratory work.

REFERENCES

Adeleke, B.S., & Babalola, O.O. 2021. Pharmacological potential of fungal endophytes associated with medicinal plants: A review. *Journal of Fungi*, 7(2), pp.1–16.

Aini, K., Elfita, Widjajanti, H., Setiawan, A., & Oktiansyah, R. 2025. Bioactivity of Secondary Metabolite of Endophytic Fungi Extract Isolated from Root of Jambu Mawar (*Syzygium jambos* (L.) Alston. *International Journal of Secondary Metabolite*, 12(1), pp. 16-32.

Akram, S., Ahmed, A., He, P., He, P., Liu, Y., Wu, Y., Munir, S., & He, Y. 2023. Uniting the Role of Endophytic Fungi against Plant Pathogens and Their Interaction. *Journal of Fungi*, 9(1). p.72.

Amalia, A., Nugraha, M.F.I., Sukenda, S., & Elya, B. 2023. In Vitro Phytochemical, Antioxidant, and Antibacterial Evaluations of Various Extracts of *Eleocharis dulcis* (Burm.f.) Trin. ex Hensch. *Tropical Journal of Natural Product Research*, 7, pp.2911–2918.

Baehaki, A., Widiastuti, I., Lestari, S., Masruro, M., & Putra, H. 2021. Antidiabetic and anticancer activity of Chinese water chestnut (*Eleocharis dulci*s) extract with multistage extraction. *Journal of Advanced Pharmaceutical Technology and Research*, 12(1), pp.40–44.

Bhuiyan, A.A., Aboul-Soud, M.A.M., Uddin, M.S., Mazumder, K., Akash, A.R., Aljowaie, R.M., Giesy, J.P., Agrawal, M., & Haque, S. 2025. GC-MS profiling, in vitro and in silico antibacterial and antioxidant potential of crude secondary metabolites of an endophytic bacterium isolated from *Enhydra fluctuans* lour. *Scientific Reports*, 15(1), pp.1–23.

Budiono, Elfita, Muharni, Yohandini, H., & Widjajanti, H. 2019. Antioxidant activity of *Syzygium samarangense* L. and their endophytic fungi. *Molekul*, 14(1), pp.48–55.

Caruso, D.J., Palombo, E.A., Moulton, S.E., & Zaferanloo, B. 2022. Exploring the Promise of Endophytic Fungi: A Review of Novel Antimicrobial Compounds. *Microorganisms*, 10(10). p.1990.

Chandimali, N., Bak, S.G., Park, E.H., Lim, H.J., Won, Y.S., Kim, E.K., Park, S.I., & Lee, S.J. 2025. Free radicals and their impact on health and antioxidant defenses: a review. *Cell Death Discovery*, 11(1), p.19.

Doifode, M.R., Hosamani, A.S., Dasgupta, D., Boruah, P., Parab, M.M., & Gupta, P.P. 2023. *Profiling of Antibacterial Compounds from Selective Medicinal Mangrove Species*. 21(1), p.11.

El-Nagar, D., Salem, S.H., El-Zamik, F.I., El-Basit, H.M.I.A., Galal, Y.G.M., Soliman, S.M., Aziz, H.A., Rizk, M.A., & El-Sayed, E.S.R. 2024. Bioprospecting endophytic fungi for bioactive metabolites with seed germination promoting potentials. *BMC Microbiology*, 24(1), pp.1–17.

Elfita, Oktiansyah, R., Mardiyanto, Widjajanti, H., Setiawan, A., & Nasution, S.S.A. 2023. Bioactive Compounds of Endophytic Fungi *Lasiodiplodia theobromae* Isolated From The Leaves of Sungkai (*Peronema canescens*). *Biointerface Research in Applied Chemistry*, 13(6), p.530.

Enyi, E.O., Chigozie, V.U., Okezie, U.M., Udeagbala, N. T., & Oko, A.O. 2025. A review of the pharmaceutical applications of endophytic fungal secondary metabolites. *Natural Product Research*, 39(11), pp.3295–3311.

Felter, S.P., Zhang, X., & Thompson, C. 2021. Butylated hydroxyanisole: Carcinogenic food additive to be avoided or harmless antioxidant important to protect food supply? *Regulatory Toxicology and Pharmacology*, 121, p.104887.

Goyal, R., Mittal, P., Gautam, R. K., Kamal, M. A., Perveen, A., Garg, V., Alexiou, A., Saboor, M., Haque, S., Farhana, A., Papadakis, M., & Ashraf, G.M. 2024. Natural products in the management of neurodegenerative diseases. *Nutrition and Metabolism*, 21(1), pp.1–15.

Gu, Y., Yang, X., Shang, C., Thao, T.T.P., & Koyama, T. 2021. Inhibitory properties of saponin from: *Eleocharis dulcis* peel against α-glucosidase. *RSC Advances*, 11(25), pp.15400–15409.

Gupta, A., Meshram, V., Gupta, M., Goyal, S., Qureshi, K.A., Jaremko, M., & Shukla, K.K. 2023. Fungal Endophytes: Microfactories of Novel Bioactive Compounds with Therapeutic Interventions; A Comprehensive Review on the Biotechnological Developments in the Field of Fungal Endophytic Biology over the Last Decade. *Biomolecules*, 13(7), p.1038.

Gurgel, R.S., de Melo Pereira, D.Í., Garcia, A.V.F., Fernandes de Souza, A. T., Mendes da Silva, T., de Andrade, C.P., Lima da Silva, W., Nunez, C.V., Fantin, C., de Lima Procópio, R.E., & Albuquerque, P.M. 2023. Antimicrobial and Antioxidant Activities of Endophytic Fungi Associated with *Arrabidaea chica* (Bignoniaceae). *Journal of Fungi*, 9(8), p.864.

Habisukan, U.H., Elfita, Widjajanti, H., Setiawan, A., & Kurniawati, A. R. 2021. Diversity of endophytic fungi in *Syzygium aqueum*. *Biodiversitas*, 22(3), pp.1129–1137.

Habisukan, U.H., Elfita, Widjajanti, H., & Setiawan, A. 2022. Secondary Metabolite and Antioxidant Activity of Endophytic Fungi Isolated from *Syzygium aqueum* Leaves Stalk. *Biointerface Research in Applied Chemistry*, 12(6), pp.7584–7595.

Hapida, Y., Elfita., Widjajanti, H., Salni. 2021. Biodiversity and antibacterial activity of endophytic fungi isolated from jambu bol (*Syzygium malaccense*). *Biodiversitas*, 22(12), pp.5668–5677.

Hashem, A.H., Attia, M.S., Kandil, E.K., Fawzi, M.M., Abdelrahman, A.S., Khader, M.S., Khodaira, M.A., Emam, A.E., Goma, M.A., & Abdelaziz, A.M. 2023. Bioactive compounds and biomedical applications of endophytic fungi: a recent review. *Microbial Cell Factories*, 22(1), pp.1–23.

Hnamte, L., Vanlallawmzuali, Kumar, A., Yadav, M.K., Zothanpuia, & Singh, P.K. 2024. An updated view of bacterial endophytes as antimicrobial agents against plant and human pathogens. *Current Research in Microbial Sciences*, 7, p.100241.

Mallik, S., Paria, B., Firdous, S.M., Ghazzawy, H.S., Alqahtani, N. K., He, Y., Li, X., & Gouda, M. M. 2024. The positive implication of natural antioxidants on oxidative stress-mediated diabetes mellitus complications. *Journal of Genetic Engineering and Biotechnology*, 22(4), p.100424.

Mendieta-brito, S., Sayed, M., Son, E., Kim, D., Marcelo, D., & Pyo, S. 2024. *Identification , Characterization , and Antibacterial Evaluation of Five Endophytic Fungi from Psychotria poeppigiana Müll . Arg ., an Amazon Plant*.

Molyneux, P. 2003. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 50, pp.211–219.

Nurnawati, E., Hutajulu, J., Widjajanti, H., & Muharni. 2019. Antimicrobial Activity of Endophytic Fungi from *Eleocharis Dulcis* (Burm.f.) Henschell). *The International MIPAnet Conference on Science and Mathematics (IMC-SciMath) 2*, pp.155–161.

Oktiansyah, R., Elfita, Widjajanti, H., Setiawan, A., Hariani, P.L., & Hidayati, N. 2023. Endophytic fungi isolated from the root bark of sungkai (*Peronema canescens*) as Anti-bacterial and antioxidant. *Journal of Medical Pharmaceutical and Allied Sciences*, 12(2), pp.5754–5761.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. 2017. Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, 2017, p. 8416763.

Pooja, G., Shweta, S., & Patel, P. 2025. Oxidative stress and free radicals in disease pathogenesis: a review. *Discover Medicine*, 2(1), p.104.

Saxena, A., & Gupta, S. 2019. Experimental comparison : Methods for the preservation of fungal cultures. *Current Research in Environmental & Applied Mycology*, 9, pp.208–212.

Selim, S., Moustafa, M.H., Almuhayawi, M.S., Gattan, H.S., Alruhaili, M.H., Alharbi, M.T., Al Jaouni, S.K., Alharbi, H.M., Kouadri, F., & Abdelaziz, A.M. 2025. Phytochemical profiling and evaluation of antioxidant, anticancer, antimicrobial and antibiofilm activities of endophytic fungi isolated from *Lavandula stricta*. *Scientific Reports*, 15(1), pp.1–14.

Slama, H.B., Chenari Bouket, A., Alenezi, F.N., Pourhassan, Z., Golińska, P., Oszako, T., & Belbahri, L. 2021. Potentials of Endophytic Fungi in the Biosynthesis of Versatile Secondary Metabolites and Enzymes. *Forests*, 12(12), p.1784.

Walsh, T.J., Hayden, R.T., & Larone, D.H. 2018. Larone's Medically Important Fungi. In *Larone's Medically Important Fungi*. https://doi.org/10.1128/9781555819880

Watanabe, T. 2010. Pictorial Atlas of Soil and Seed Fungi. In *Pictorial Atlas of Soil and Seed Fungi*. https://doi.org/10.1201/ebk1439804193

Wen, J., Okyere, S.K., Wang, S., Wang, J., Xie, L., Ran, Y., & Hu, Y. 2022. Endophytic Fungi: An Effective Alternative Source of Plant-Derived Bioactive Compounds for Pharmacological Studies. *Journal of Fungi*, 8(2), p.205.

Yang, J., Tang, X., Shuai, L., Soo, Y., & Jo, M. 2020. Chemical characterization , antioxidant properties and anti-inflammatory activity of Chinese water chestnut extracts. *ScienceAsia*, 46, pp.151–156.

You, Y.H., Park, J.M., Park, J.H., & Kim, J.G. 2015. Diversity of endophytic fungi associated with the roots of four aquatic plants inhabiting two wetlands in Korea. *Mycobiology*, 43(3), pp.231–238.

Zakariyah, R.F., Ajijolakewu, K.A., Ayodele, A.J., Folami-A,B.I., Samuel, E.P., Otuoze, S.O., Abdulrauf, L.B., & Ahmed, R.N. 2024. Progress in endophytic fungi secondary metabolites: biosynthetic gene cluster reactivation and advances in metabolomics. *Bulletin of the National Research Centre*, 48(1), p.44.