Life Science and Biotechnology
Volume 2, Number 2, November 2024
Pages: 49 - 57

# Bioactivity of Endophytic Fungal Extract Isolated from Purun (*Eleocharis dulcis* (Burm.f.) Trin. ex Hensch)

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## Abstract

The study investigates the bioactivity of endophytic fungi isolated from *Eleocharis dulcis* (Burm.f.) Trin. ex Hensch., focusing on their potential as alternative sources of bioactive compounds. *E. dulcis*, a plant commonly found in wetland ecosystems, has traditionally been used for its medicinal properties. However, the massive extraction of the plant may lead to ecological imbalance, prompting the exploration of endophytic fungi as a sustainable alternative. The objective of this research is to isolate, identify, and evaluate the antimicrobial and antioxidant properties of endophytic fungi from *Eleocharis dulcis*. Endophytic fungi were isolated aseptically, followed by morphological identification. Antioxidant and antimicrobial assays were conducted to assess the bioactivity of the fungal extracts. Antioxidant activity was evaluated using the DPPH method, while antibacterial activity was assessed using the disc paper diffusion method. The results revealed that several isolates (from leaves: LP1 – LP6, from root: RP1 – RP3) exhibited significant antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, with *Penicillium spp.* and *Cladosporium spp.* showing the highest levels of bioactivity. Additionally, all fungal isolates demonstrated very strong antioxidant activity, with most achieving an $IC_{50}$ below 20 µg/mL. In conclusion, the findings suggest that endophytic fungi from *E. dulcis* represent a valuable source of bioactive compounds with potential applications in pharmaceuticals. Further research is required to isolate and characterize the active compounds, as well as to evaluate their efficacy through in vivo studies.

**Keywords**: Antibacterial, Antioxidant, *Eleocharis dulcis*, Endophytic fungi

## Introduction

Purun (*Eleocharis dulcis (Burm.f.)* Trin. ex Hensch.), commonly known as Chinese water chestnut, is a perennial aquatic plant commonly found in wetlands and marshy areas. It plays a significant role in wetland ecosystems by stabilizing the soil, preventing erosion, and maintaining water quality through nutrient absorption and sediment trapping (Baehaki et al., 2021; Lu et al., 2022). This plant contributes to biodiversity, offering habitat and food sources for various aquatic species. Its ability to thrive in waterlogged conditions makes it a vital component in flood control and wetland conservation efforts (Alikhani et al., 2021; Cantonati et al., 2020; Schofield et al., 2019). Ecologically, Purun acts as a biofilter, mitigating the impact of pollutants by absorbing heavy metals and organic compounds from the water. This natural filtration system enhances aquatic ecosystem health, establishing Purun as essential for wetland management. The loss of such plants could disrupt the balance of these ecosystems, leading to habitat degradation and a decline in biodiversity (Pachaiappan et al., 2022; Zhang et al., 2023).

In addition, Purun has been utilized in traditional medicine, particularly in East and Southeast Asia. The rhizomes and shoots of Purun are rich in secondary metabolites such as flavonoids, phenolic acids, and terpenoids, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. These bioactive compounds make Purun valuable in folk medicine for treating ailments such as digestive issues, skin disorders, and inflammation. Research on the secondary metabolites of Purun has revealed its potential in modern pharmacology. The plant's extracts have demonstrated therapeutic properties, including antibacterial and antifungal activities (Asikin &

Thamrin, 2012; Baehaki et al., 2021; Li et al., 2023; Ramadhani et al., 2020; Zhan et al., 2014). These findings support its long-standing use in traditional medicine, highlighting the medicinal potential of Purun beyond its ecological importance. Furthermore, the presence of unique polysaccharides in Purun has been identified as having immunomodulatory effects, enhancing the plant's value in developing nutraceuticals (Chen et al., 2022; Di Sotto et al., 2020; Do Socorro Chagas et al., 2022). The natural compounds found in Purun thus offer promising avenues for both health-related products and sustainable agricultural practices. These distinctive properties emphasize Purun's role as an underutilized plant with significant potential for further research and development. However, massive use of purun can disrupt its ecological function, so alternatives are needed to meet the need for materials for medicines, namely endophytic fungi.

Endophytic fungi isolated from the plant present an alternative and potentially more sustainable source of bioactive compounds. Endophytic fungi are microorganisms that reside within plant tissue without causing harm (Anand et al., 2023; Digra & Nonzom, 2023). These fungi often produce secondary metabolites similar to those of their host plants, sometimes with enhanced bioactivity ( Habisukan et al., 2021ab), 2021; Sagita et al., 2021; Srinivasa et al., 2022; Torres-Mendoza et al., 2020). By utilizing endophytic fungi, it is possible to harness these bioactive compounds without harvesting the plant, preserving its ecological role in wetland ecosystems. Moreover, cultivating endophytic fungi in laboratory conditions allows for a more controlled and scalable production of bioactive compounds. This method reduces the environmental impact of plant extraction and ensures the conservation of Purun's natural habitats (El-Sayed et al., 2020). Therefore, the use of endophytic fungi provides a promising solution to balancing the need for bioactive compounds with the preservation of ecologically important species like Purun.

Several studies have demonstrated that endophytic fungi exhibit superior bioactivity compared to their host plants. For instance, endophytic fungi isolated from medicinal plants have been shown to produce higher concentrations of secondary metabolites with potent antimicrobial and anticancer properties (Budiyanto et al., 2022; Elfita et al., 2023; Gu et al., 2022; Oktiansyah et al., 2024). Research on fungal isolates from *Eleocharis* species revealed that some endophytes produced more effective antifungal and antibacterial agents than the plant itself, suggesting a valuable alternative for drug discovery (Nurnawati et al., 2022). Furthermore, endophytic fungi have exhibited a broad spectrum of bioactivities, including antioxidant, anti-inflammatory, and cytotoxic effects, making them a rich source of pharmacologically relevant compounds. The ability of these fungi to produce novel compounds, which may not be present in their host plants, highlights their potential for developing new drugs and therapies. This study aims to evaluate the bioactivity of endophytic fungi from *E. dulcis*.

## Material and Methods

### Isolation of Endophytic Fungi from *E. dulcis*

Fresh plant samples, including roots and leaves, were collectedand carefully washed under running water to isolate endophytic fungi from *E. dulcis*. The samples (root and leaves) were then cut into small 1-2 cm segments using sterile tools in a laminar flow hood. For surface sterilization, the plant segments were immersed in 70% ethanol for 1 minute, followed by treatment with 2.5% sodium hypochlorite for 3-5 minutes to eliminate surface microorganisms. After sterilization, the tissues were rinsed thoroughly with sterile distilled water multiple times to ensure complete removal of sterilizing agents. The sterilized plant segments were placed on Potato Dextrose Agar (PDA) plates, which are supplemented with antibiotics such as streptomycin (25 µg/mL) to prevent bacterial contamination. The plates were incubated at 25-28°C in the dark for 7-14 days, during which the fungal growth emerging from the plant tissues was monitored. Fungi growing from the inner tissue were subcultured and purified for further identification (Oktiansyah et al., 2023).

### Morphological Identification of Endophytic Fungi Isolated from *E. dulcis*

Morphological identification of endophytic fungi from *E. dulcis* was conducted by observing fungal growth characteristics on Potato Dextrose Agar (PDA) plates after seven days of incubation. Key features documented included colony color and texture. The texture ranged from cottony and velvety to smooth, while the colony colors varied from white, green, and black to more pigmented shades, depending on the fungal species.

Microscopic examination followed to observe reproductive structures. A small portion of the mycelium was placed on a microscope slide, stained with lactophenol cotton blue, and examined under a light microscope. Key features, including the size, shape, and arrangement of conidia (spores), sporangia, and hyphal structures, were carefully recorded. These included the presence of septate or non-septate hyphae, fruiting bodies, and spore morphology (e.g., unicellular or multicellular, smooth or ornamented), all of which provide critical taxonomic information. Combined with colony characteristics, these morphological traits allowed for preliminary fungal identification (Walsh et al., 2018; Watanabe, 2010).

### Extraction of Endophytic Fungi

Purified fungal isolates (nine in total) were inoculated into Potato Dextrose Broth (PDB) and incubated under shaking conditions to promote fungal growth for four weeks. After cultivation, the fungal biomass was separated from the culture medium through filtration. The liquid culture was then macerated with ethyl acetate as a solvent for seven days. The mixture was filtered, and the solvent evaporated to yield a concentrated fungal extract ( Habisukan et al., 2021a).

### Antioxidant Activity Test

The antioxidant activity was assessed through the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Extracts at concentrations of 1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL (performed in triplicate) were combined with 3.8 mL of 0.5 mM DPPH solution in 0.2 mL aliquots and incubated for 30 minutes in darkness. Ascorbic acid was used as positive control. The reduction in absorbance was recorded at 517 nm using a UV-Vis spectrophotometer. The percentage of radical scavenging activity was determined using the following formula (Molyneux P, 2003):

$$\% \text{ Inhibition} = \frac{A_k - A_s}{A_s}$$

$A_k$ = Absorbance of control
$A_s$ = Absorbance of samples

### Antibacterial Activity Test

The antibacterial activity was tested using the disk diffusion technique. Paper disks (6 mm in diameter) were saturated with fungal extracts (400 µg/mL) and positioned on Muller-Hinton Agar (MHA) plates previously inoculated with bacterial strains. The plates were incubated at 37°C for 24-48 hours. Zones of inhibition around the disks were measured to assess antimicrobial activity. Tetracycline (30 µg/disk) served as the positive control, while ethyl acetate was used as the solvent control (Hapida et al., 2021).

## Results and Discussion

### Morphological Identification of Endophytic Fungi

Endophytic fungi were successfully isolated and identified from *E. dulcis*, yielding six isolates from the leaves (LP1–LP6) and three isolates from the roots (RP1–RP3). Identification was conducted based on their morphological characteristics, as detailed in Tables 1 and 2. Each isolate exhibited unique morphological features, as illustrated in Figure 1.

Table 1. Colony Characteristics of Endophytic Fungi from *E. dulcis*

| Isolates | Surface Colony | Reverse Colony | Structure | Pattern | Elevation |
|---|---|---|---|---|---|
| LP1 | Black | White | Powdery | Spread | Umbonate |
| LP2 | Green | White | Powdery | Spread | Umbonate |
| LP3 | Black | White | Powdery | Spread | Umbonate |
| LP4 | Green | White | Velvety | Zonate | Raised |
| LP5 | Black | White | Powdery | Spread | Umbonate |
| LP6 | White | White | Cottony | Zonate | Raised |
| RP1 | Black | White | Powdery | Spread | Umbonate |
| RP2 | Green | Brownish Orange | Velvety | Zonate | Raised |
| RP3 | Greenish Brown | Black | Velvety | Zonate | Raised |

The morphological analysis of fungal isolates from *E. dulcis* revealed significant differences in colony characteristics and spore structures (Tables 1 and 2). The colonies exhibited a variety of features, including color, texture, and structure, which reflect the fungi's environmental adaptability. For example, isolates identified as Aspergillus niger (LP1, LP3, RP1) displayed black, powdery colonies with umbonate elevation, suggesting potential adaptations to saline conditions. Conversely, LP6 (*Cylindrocarpon* sp.) formed cottony colonies with cylindrical spores, indicating a distinct ecological role or specific adaptations. Variations in colony elevation, ranging from umbonate in LP1 to raised in LP4, further demonstrated the morphological diversity among the isolates.

Table 2. Microscopic Characteristics of Endophytic Fungi from *E. dulcis*

| **Isolates** | **Spore** | **Shape** | **Hyphae** | **Characteristic** | **Species** |
|---|---|---|---|---|---|
| LP1 | Conidia | Globose | Septate | Septate hyphae, conidiophores long, smooth, may be brownish near top. | *Aspergillus sp.* |
| LP2 | Conidia | Globose | Septate | Hyphae are septate and branched or unbranched conidiophores that have secondary branched. | *Penicillium spp* |
| LP3 | Conidia | Globose | Septate | Septate hyphae, conidiophores long, smooth, may be brownish near top. | *Aspergillus sp.* |
| LP4 | Conidia | Globose | Septate | Septate hyphae, conidiophores smooth, and large. | *Aspergillus sp.* |
| LP5 | Conidia | Globose | Septate | Septate hyphae, conidiophores long, smooth, may be brownish near top.. | *Aspergillus sp.* |
| LP6 | Conidia | Cylindrical | Septate | Conidia phialosporus, terminal, of two kinds, | *Cylindrocarpon sp.* |
| RP1 | Conidia | Globose | Septate | Septate hyphae, conidiophores long, smooth, may be brownish near top. | *Aspergillus sp.* |
| RP2 | Conidia | Globose | Septate | Conidiophores smooth, short, and brown, darkening with age. | *Aspergillus sp.* |
| RP3 | Conidia | Round to oval | Septate | Conidiophores are dark and branched, very in length | *Cladosporium spp* |

The microscopic examination provided additional evidence of this diversity. Most isolates produced globose or cylindrical spores with septate hyphae. For instance, *Aspergillus calvatus* (LP4) and the *Aspergillus nidulans* complex (RP2) exhibited smooth, large conidiophores, consistent with their established ecological roles in spore dispersal. In contrast, *Cladosporium* spp. (RP3) produced round to oval spores with branched conidiophores, indicative of a distinct interaction with the host plant. These unique microscopic features underscore the complex relationships between the fungi and their host, potentially contributing to their ecological functions.

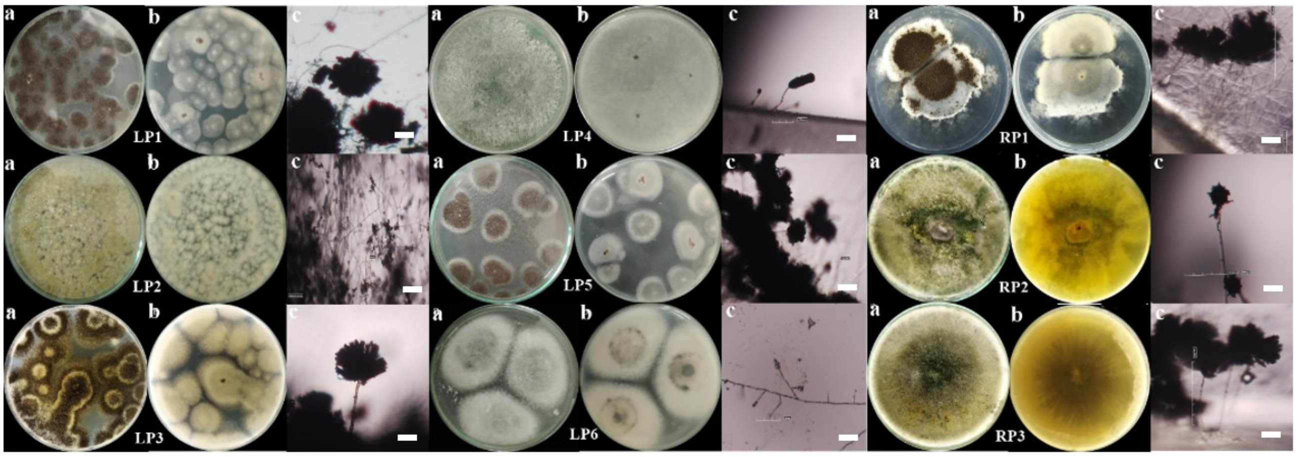


Figure 1. Characteristics of endophytic fungi from *E. dulcis* (scale bar: 50µm; 7 days old on PDA media; a: front view; b: reverse view; c: microscopic view)

The morphological analysis of fungal isolates from *E. dulcis* revealed notable differences in colony features and spore structures (Table 1 and 2). The colonies exhibited diverse characteristics such as color, texture, and structure, reflecting the environmental adaptability of these fungi. For instance, isolates of *Aspergillus niger* (LP1, LP3, RP1) were characterized by black, powdery colonies with umbonate elevation, indicating a possible adaptation to saline environments. On the other hand, LP6 (*Cylindrocarpon sp.*) displayed a cottony colony structure with cylindrical spores, suggesting a distinct ecological role or adaptation. The variation in colony elevation, ranging from umbonate in LP1 to raised in LP4, demonstrates the morphological diversity among the isolated fungi.

Microscopic examination further confirmed this diversity. Most isolates produced globose or cylindrical spores with septate hyphae. For example, *Aspergillus calavatus* (LP4) and *Aspergillus nidulans complex* (RP2) exhibited smooth, large conidiophores, consistent with their known ecological roles in spore dispersal. In contrast, *Cladosporium spp.* (RP3) had round to oval spores and branched conidiophores, suggesting a different interaction with the host plant. These distinct microscopic features highlight the complex relationships between the fungi and their host, which may play a role in their ecological functions.

**Bioactivity of Endophytic Fungi Extract Isolated from *E. dulcis***

The isolated endophytic fungi from *E. dulcis* exhibited a range of antibacterial and antioxidant properties (Table 3). Several fungal extracts demonstrated very strong antioxidant activity ($IC_{50}$ < 20 µg/mL) and robust antibacterial activity (⩾ 70%). Among these, the extract from isolate LP2 and RP3 showed the highest level of bioactivity compared to other isolates.

Table 3. Antibacterial and Antioxidant Activities of Endophytic Fungal Extracts from *E. dulcis*

| Samples | Antibacterial Activity (%) | | Antioxidant Activity (µg/mL) |
|---|---|---|---|
| | *E.coli* | *S.aureus* | |
| LP1 | 0 | 30,422* | 47,829*** |
| LP2 | 88,698*** | 59,34** | 11,415**** |
| LP3 | 38,564* | 28,624* | 17.388**** |
| LP4 | 56,272** | 52,414** | 19.366**** |
| LP5 | 54,478** | 38,43* | 12.058**** |
| LP6 | 59,382** | 43,544* | 13.751**** |
| RP1 | 52.472** | 52.326** | 18.121**** |
| RP2 | 68.626** | 40.162* | 14.345**** |
| RP3 | 86.194*** | 49.548* | 13.576**** |
| Methanol Extract of leaves | 57.34** | 54.664** | 19.674**** |
| Methanol Extract of root | 40.378* | 0 | 48.250*** |
| Positive control | Tetracycline 100*** | Tetracycline 100*** | Ascorbic acid 10.08**** |

Antibacterial activity percentage: *** ≥ 70% (strong), **50-70% (moderate), and *< 50% (weak); Antioxidant activity $IC_{50}$ (µg/mL): ****very strong <20 µg/mL ***strong < 100 µg/mL; **moderat 100-500 µg/mL; * weak > 500 µg/mL

The bioactivity test revealed significant differences in antibacterial and antioxidant activities among the isolates (Table 3). Isolates such as LP2 (*Penicillium spp.*) and RP3 (*Cladosporium spp.*) showed strong antibacterial activity against *E. coli*, with inhibition rates of 88.698% and 86.194%, respectively, indicating their potential for use in antimicrobial applications. In contrast, LP1 (*Aspergillus niger*) exhibited no activity against *E. coli* but showed notable antioxidant activity, suggesting its role in oxidative stress management rather than antibacterial defense. In terms of activity against *S. aureus*, the isolates exhibited varying degrees of inhibition with no strong categories. LP2 showed the lowest activity and RP3 displayed moderate inhibition. The antioxidant activities across all isolates were consistently high. The very strong antioxidant activities were observed in all isolates ($IC_{50}$ < 20 µg/mL), except LP1 ($IC_{50}$ < 100 µg/mL). It is suggested that these fungal species may contribute significantly to the oxidative stress response of *E. dulcis*, which is known for thriving in challenging environments.

Overall, the findings provide the antibacterial activity of endophytic fungi play within *E. dulcis*, highlighting their potential applications in medicine and agriculture. The diversity in their morphological traits and bioactive properties suggests that these fungi might contribute to the host plant's defense mechanisms and stress tolerance. Further research is needed to isolate the active compounds from promising isolates such as LP2 and RP3 for potential use in pharmaceutical and biotechnological fields. Based on morphological identification, LP2 and RP3 were *Penicillium spp*. and *Cladosporium spp*., respectively.

The endophytic fungus *Penicillium spp.* and *Cladosporium spp.* demonstrated remarkable antibacterial activity against the Gram-negative bacterium *Escherichia coli* and exhibited potent antioxidant properties, both of which are key indicators of its biotechnological potential. The superior antibacterial activity observed in the endophytic fungi against *E. coli* can be attributed to several factors, including the production of bioactive secondary metabolites. *Penicillium* species are known for synthesizing a wide range of bioactive compounds, such as penicillin, alkaloids, and polyketides, which have been extensively studied for their antimicrobial properties while *Cladosporium* is known to produce a variety of secondary metabolites that contribute to their bioactivity, including phenolic compounds, terpenoids, and alkaloids, which possess significant antimicrobial and antioxidant properties (Fadhillah et al., 2019; Fang et al., 2019; Grijseels et al., 2016; Habisukan et al., 2021b); Ju et al., 2022; Masoudi Khorasani et al., 2023; Wen et al., 2023). The presence of such compounds plays a crucial role in disrupting bacterial cell membranes and interfering with bacterial metabolic processes, ultimately leading to cell death. These compounds likely disrupt the integrity of the bacterial cell membrane, impair metabolic functions, or inhibit protein synthesis in *E. coli*, leading to cell death (Abdelhakim et al., 2022; Miklasińska-Majdanik et al., 2018; Nguyen & Bhattacharya, 2022; Oteiza et al., 2005; Wang et al., 2018). Given the resilience of Gram-negative bacteria, which possess a complex outer membrane that offers resistance to many antibiotics, the strong antibacterial effects of *Penicillium spp.* suggest that its metabolites effectively penetrate or disrupt this protective barrier.

In addition to its antibacterial properties, *Penicillium spp.* and *Cladosporium spp.* also exhibited very strong antioxidant activity, which is crucial for neutralizing harmful free radicals and preventing oxidative stress. The antioxidant capacity of *Penicillium spp.* . and *Cladosporium spp.* is primarily due to the production of antioxidant metabolites, such as phenolic and flavonoid, which can scavenge reactive oxygen species (ROS) and reduce oxidative damage in cells (Ebadi et al., 2024; Fadhillah et al., 2019; Muthukrishnan et al., 2022; Tang et al., 2021; Verma et al., 2022). These compounds play a critical role in protecting the host plant from environmental stressors such as UV radiation, pollution, and pathogen attacks by mitigating oxidative damage (Asiminicesei et al., 2024; Rahman et al., 2022; Stiller et al., 2021). For instance, phenolic compounds are known for their ability to donate electrons or hydrogen atoms to neutralize free radicals, thereby preventing cellular damage (Parcheta et al., 2021).

The dual action of *Penicillium spp.* (LP2) and *Cladosporium spp.* (RP3) as both an antioxidant and antibacterial agent may be explained by the interconnectedness of oxidative stress and microbial defense. The presence of antioxidants in the fungal extracts helps maintain the stability and activity of its bioactive compounds, which may otherwise degrade under oxidative conditions. Additionally, the antioxidant metabolites themselves may enhance the antibacterial action by limiting the oxidative stress response in the host, thereby boosting the overall efficacy of the fungal extracts. This synergy between antioxidant and antibacterial activities highlights the ecological importance of *Penicillium spp.* in maintaining the health and resilience of its host plant, as well as its potential applications in pharmacology and agriculture. The results of this study are very useful as a fundamental for thinking about further research, such as isolating the pure compound using the NMR method and further in vivo testing to see its direct effect on the body functions of experimental animals.

## Acknowledgments

We would like to thank the biology laboratory, Integrated Laboratory, Universitas Islam Negeri raden Fatah for facilitating this research.

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