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Screening and evaluation of antidiabetic activities of endophytic fungi associated with *Etlingera elatior*

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Abstract. *Nurjannah L, Azhari A, Wulandari AP, Amin S, Supratman U. 2023. Screening and evaluation of antidiabetic activities of endophytic fungi associated with* Etlingera elatior. *Biodiversitas 24: 3481-3487. Etlingera elatior* (Jack) R.M.Sm. has been well-documented to have antioxidant and antidiabetic properties. Among all medical conditions worldwide, diabetes mellitus is one of the leading causes of death. Therefore, research on the discovery of antidiabetic medicines inhibitory to α-glucosidase (an enzyme for degrading complex dietary carbohydrates into sugar in the digestive process) is urgently needed. This study aims to isolate and evaluate the potential of the fermentation culture of endophytic fungi associated with *E. elatior* to inhibit the activity of α-glucosidase. The endophytic fungi were isolated from various parts of *E. elatior*. A total of 29 isolates were successfully isolated from the plant's roots, stems, leaves, and flowers. The endophytic fungi were tested for their ability to inhibit α-glucosidase activity in bioassays. Among those isolates, 8 were inhibitory to such an enzyme, and 2 showed the highest inhibitory activities. Morphotype and molecular identification of those isolates, using their Internal Transcribed Spacer (ITS) regions of ribosomal DNA (rDNA), identified them as *Daldinia eschscholtzii* isolate CFL 7 and *Hypoxylon trugodes* voucher YMJ 57, with IC_{50} values of 738 µg/mL and 825 µg/mL, respectively. That indicates that they are potentially used in preventing or treating diabetic mellitus.

Keywords: α-glucosidase, antidiabetic, endophytic fungi, *Etlingera elatior*

Abbreviations: ITS: Internal Transcribed Spacer, rDNA: ribosomal DNA

INTRODUCTION

Diabetes is one of the many major risks of mortality in the world. According to the International Diabetes Federation (IDF 2019), 1 in 11 adults aged 20-79 worldwide suffered from diabetes (around 463 million). However, 50% of them (232 million) did not know they had diabetes. Recently, Indonesia has been on the 6th rank in the world after China, India, the United States, Brazil, and Mexico, in terms of people aged 20-79 years suffering from diabetes (around 10.3 million people) (Health Research and Development Agency 2019).

Diabetes could be prevented or controlled by managing monosaccharide absorption or controlling the activities of carbohydrate-degrading enzymes (Alagesan et al. 2012). Two principal digestive enzymes, α-amylase and α-glucosidase are responsible for degrading complex dietary carbohydrates into sugar in the digestive tract (Lee et al. 2013). In recent years, plants have been utilized as traditional medicines to treat patients with various types of diabetes, such as retinopathy, peripheral neuropathy, diabetes, etc.

The application of endophytes, however, has not been extensively explored for antidiabetic agents (Franco et al. 2002). Exploration of microbe valuable bioactive compounds is relatively easier than plant bioactive compounds and tends to be inexpensive (Nair et al. 2013). Different types of plants are known to produce α-amylase and α-glucosidase inhibitors, but reports on such inhibitors from endophytic microorganisms are limited (Mun'im et al. 2013). The use of α-glucosidase inhibitors in the therapy of diabetic patients is safer than that of other oral antidiabetic drugs. Therefore, recent research has been focused on finding sources of α-glucosidase inhibitors. According to Kaur et al. (2018), endophytic fungi have great potential as a source of α-glucosidase inhibitory compounds.

Etlingera elatior (Jack) R.M.Sm. belongs to the Zingiberaceae family and has been used as an antidiabetic

(Wijekoon et al. 2011). Many studies have shown that parts of this plant (the leaves, rhizomes, flowers, and seeds) have been used as sources of antioxidants. Chan et al. (2011) reported that this plant's leaves had an eight times higher antioxidant capacity than the rhizomes. That was assumed to be related to the phenolic compounds in these plant parts (Ghasemzadeh et al. 2015). There have been limited reports on the antidiabetic test on the endophytic fungi of *E. elatior*. Traditionally, *E. elatior* has been used to cure fever, cough, and ear infections and improve postpartum maternal fitness; it has also been discovered to exhibit healing, anti-hypertensive, and antidiabetic properties (Silalahi 2017).

Expansive research has also been carried out to study the pharmacological activities of *E. elatior* using various methods. Nor et al. (2020), for example, found that the flowers of *E. elatior* exhibited a high level of inhibitory activity against α-amylase (99.70±2.88%) and a moderate level of inhibitory activity against α-glucosidase (52.39± 1.50%). Additionally, the plant's rhizome crude extracts exhibited good inhibition against both α-glucosidase and α-amylase in the ranges of 28.36-99.79% and 35.91-58.13%, respectively, when applied at a concentration of 25 µg/mL (Srey et al. 2014). Active compounds such as flavonoids, phenolics, and saponins, which could inhibit amylase and glucosidase, neutralize free radicals, and protect against pancreatic beta cell damage in antihyperglycemic activity in diabetic patients, were detected in the *E. elatior* plant (Juwita et al. 2018; Putri 2020).

Ethanol extract from *E. elatior* leaves had a blood glucose-lowering effect on alloxan-induced diabetic rats (Fitrianita 2018). Among the family of Zingiberaceae, *E. elatior* (particularly in its leaves) is known to have the strongest antioxidant activity (Srey et al. 2014). According to Chan et al. (2007), chlorogenic acid compounds and the flavonoid quercetin are the dominant compounds in the plant's leaf extract. Chlorogenic acid is known to be involved in glucose metabolism, while the flavonoid quercetin has the effect of lowering blood glucose levels due to its antioxidant abilities (Chan 2009; Meng et al. 2013).

Research on endophytic fungi isolated from medicinal plants, such as *E. elatior*, has been limited recently. Medicinal plants have been reported as potential sources of endophytic fungi capable of producing bioactive compounds (Ginting et al. 2013; Sofian et al. 2021; Nurjannah et al. 2023). Endophytic fungi also produce new bioactive compounds with pharmacological or non-pharmacological activity (Shiono et al. 2013; Azhari and Supratman 2021; Nakamura et al. 2021; Supratman et al. 2021). This research aims to investigate and evaluate the presence of antidiabetic properties exhibiting endophytic fungi by isolating and screening such endophytic fungi from healthy and symptom-free tissues of *E. elatior*. Evaluation of their antidiabetic activity based on the inhibition of α-glucosidase was also conducted in the present research.

MATERIALS AND METHODS

Plant materials

Samples of *E. elatior* (roots, leaves, stems, and flowers) were collected from healthy plants at the Arboretum of Padjadjaran University, West Java, Indonesia. The roots, leaves, stems, and flowers of *E. elatior* were inserted into clean polyethylene bags, immediately brought to the laboratory, and washed with water. The initial determination of the plant species was carried out at the Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

Sample sterilization and isolation of endophytic fungi

Sterilization was conducted using the modified method of Wulandari et al. (2022). *Etlingera elatior* samples were washed with running water for 10 minutes to remove dust, dried at ambient temperature on sterile filter paper in a laminar airflow, and sterilized on the surface by immersion in 70% ethanol for 1 minute, in 5.3% sodium hypochlorite for 5 minutes, and in 70% ethanol for 30 seconds, and rinsed with distilled water for 1 minute. Isolation of endophytic fungi was carried out using a direct plating method. Each sample of plant organ was cut (in the size of 1 × 1 × 0.5 cm), and the cuts were placed in Petri dishes containing PDA supplemented with chloramphenicol as an antibacterial (50 mg/L). For control, some distilled water for rinsing was poured (1 mL) into a chloramphenicol-supplemented PDA medium. Only endophytic fungi that grew in the samples were isolated and transferred onto fresh growing media plates using the streak technique. The grown fungi were confirmed as endophytes because the surface sterilization method was previously conducted, and no colonies grew in the PDA control that pour with previously rinsed distilled water. Single colonies were transferred from the cultures to a slant agar medium in test tubes and used as stock cultures for further experiments. These pure cultures were kept at 4°C until it was required.

Fermentation and extraction of endophytic fungi

Every single colony of endophytic fungi was rejuvenated by growing it on Potato Dextrose Agar (PDA), HiMedia®, and incubated at 28°C for 7 days. After this incubation period, a few hyphae of endophytic fungal isolates were inoculated into 50 mL of liquid fermentation medium of Potato Dextrose Broth (PDB) and incubated at ambient temperature (an average of 28°C) for 28 days (Yan et al. 2020). The medium and mycelia were blended, and the liquid was extracted three times in ethyl acetate. These clear extracts were taken, concentrated in a rotary evaporator, and weighed (Azhari et al. 2023).

Alpha-glucosidase inhibition assay

The metabolite extracts were evaluated for α-glucosidase inhibitory activity according to the method specified in Sancheti et al. (2009) with slight modifications. The reaction mixture consisted of 50 µL of 0.1 M phosphate buffer (pH 7.0), 25 µL of 0.5 mM 4-nitrophenyl α-D-glucopyranoside (dissolved in 0.1 M phosphate buffer,

pH 7.0), 2,000 µg mL^{-1} of the sample, and 25 µL of α-glucosidase (Sigma) (a stock solution of 1 mg mL^{-1} in 0.01 M phosphate buffer, pH 7.0 was diluted at 0.04 units mL^{-1} with the same buffer, pH 7.0 just before assay). This reaction mixture was then incubated at 37°C for 30 min. The reaction was terminated by adding 100 µL of 0.2 M sodium carbonate solution. Enzymatic hydrolysis of the substrate was conducted by monitoring the p-nitrophenol released in the reaction mixture at 410 nm using a microplate reader. All experiments were carried out in triplicate, and the calculation of the enzyme activity was based on the following equation:

$$\% \text{ Inhibition} = \frac{[(\text{Absorbance (Control)} - \text{Absorbance (Sample)}]}{\text{Absorbance (Control)}} \times 100\%$$

One unit of enzyme is defined as the amount of enzyme (α-glucosidase) required for the formation of one µmol of the product (p-Nitrophenol) from the substrate (p-nitrophenyl-α-D-glucopyranoside) per minute. The IC_{50} value, indicating the sample concentration in a test capable of inhibiting α-glucosidase at 50%, was obtained by making a linear curve showing the relationship between the concentration of the test solution (x-axis) and percentage (%) inhibition activity (y-axis). Different extract concentrations were used (625, 1,250, 2,500, 5,000, and 10,000 ppm).

Morphotype identification

Macroscopic observation parameters of microbial morphology were based on Roosheroe et al. (2006). These include the colors of the front and backside colony surfaces, the presence of a radial line, the presence of concentric shapes forming a circle on the inside of the colony, and the surface texture (Schulz and Boyle 2005).

Molecular identification of potential endophytic fungi

The procedures of genomic DNA extraction adopted the method specified in Ramona et al. (2023) with slight modification. The fungal DNAs were extracted with Quick-DNA Fungal Miniprep Kit (Zymo Research, D6005). Endophytic fungi at 50-100 mg (wet weight) suspended in 200 µL of water or isotonic buffer (PBS) were added into a ZR BashingBead™ Lysis Tube (0.1 and 0.5 mm), added with 750 µL of BashingBead™ Buffer, secured in a bead beater fitted with a 2 mL tube holder assembly, and processed at a maximum speed for 5 minutes. This was followed by centrifugation of the ZR BashingBead™ Lysis Tube (0.1 and 0.5 mm) in a microcentrifuge at 10,000 x *g* for 1 minute, and 400 µL of the supernatant produced was transferred into a Zymo-Spin™ III-F Filter in a collection tube, centrifuged at 8,000 x *g* for 1 minute. The supernatant was added with 1,200 µL of Genomic Lysis Buffer in the collection tube. A volume of 800 µL of this mixture was transferred into a Zymo-Spin™ IICR Column 3 in a collection tube, centrifuged at 10,000 x *g* for 1 minute, and discarded. The flow through from the collection tube and the previous steps were repeated. Then, 200 µL of DNA Pre-Wash Buffer was added into the Zymo-Spin™ IICR Column in a new collection tube and centrifuged at 10,000 x *g* for 1 minute. This was followed by adding 500 µL of g-DNA Wash Buffer into the Zymo-Spin™ IICR Column. It was centrifuged at 10,000 x *g* for 1 minute and transferred into a clean 1.5 mL microcentrifuge tube, added with 100 µL (35 µL minimum) of DNA Elution Buffer directly to the column matrix. The last step was centrifugation at 10,000 x *g* for 30 seconds to elute the DNA.

Molecular identification was carried out using the molecular marker Internal Transcribed Spacer (ITS). The stages of DNA amplification were conducted in 25 µL of PCR master mix containing 9.5 µL of alkaline free water, 12.5 µL of 2x My Taq HS Red Mix (Bioline, BIO-25048), 1 µL of DNA template, 1 µL of 10 µM ITS 1 Primer, and 1 µL of 10 µM ITS 4 Primer. The primers used were ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') as the forward primer and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3') as the reverse primer. The reactions in the amplification process were carried out in 35 cycles in 3 stages, namely: pre-denaturation at 95°C for 3 minutes, denaturation at 95°C for 15 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 45 seconds, and final extension at 72°C for 3 minutes. The PCR products were species barcoding fungi (~ 700 bp). The PCR products were analyzed by 1% gel electrophoresis. The PCR products were sent for sequencing to the 1st BASE in Malaysia, Apical Scientific Sdn Bhd. The results of DNA sequencing were aligned with their counterparts (Basic Local Alignment Search Tool (BLAST)) deposited at the National Centre for Biotechnology Information (NCBI).

Statistical analysis

All measurements were carried out in triplicate (n = 3). All results were presented in means ± Standard Deviations (SD), calculated using Microsoft Excel software. The data of α-glucosidase inhibition were analyzed using a one-way Analysis of Variance (ANOVA) with IBM SPSS Statistics 22 software. When significant differences $p > 0.05$ were indicated, the data were further analyzed with the Tukey HSD test.

RESULTS AND DISCUSSION

In vitro α-glucosidase inhibitory activity

Twenty-nine endophytic fungi were successfully isolated from *E. elatior*, including those from the roots (code: RT, 9 isolates), leaves (code: LV, 7 isolates), flowers (code: FL, 5 isolates), and stems (code: ST, 6 isolates) before examination for potential α-glucosidase inhibitory activity, fermentation, and crude ethyl acetate extraction were performed.

The results of the enzymatic activity assays show that only 8 isolates were found to be inhibitory to such an enzyme, and two of them showed the highest inhibitory activities (see Table 1). Although the plant's roots contributed the highest number of endophytic fungi in our research, none of those endophytic fungi demonstrated activity as a potential antidiabetic fungal candidate.

Table 1. The percentages of α-glucosidase inhibition of isolates of the endophytic fungi in *Etlingera elatior*

	Codes of isolates	% α-Glucosidase inhibition*
Stems	ST-2D	29.045 ± 2.042
	ST-2F	28.289 ± 0.362
	ST-1A	26.309 ± 0.276
	ST-7I	25.639 ± 0.308
Flowers	FL-6F	29.215 ± 0.628
Leaves	LV-1B	3.863 ± 0.055
	LV-1C	61.526 ± 0.399**
	LV-1A	69.764 ± 0.308**

Note: * Values in Table 1 (average)±standard deviation of triplicate experiments (n = 3). ** The two highest levels of α-glucosidase inhibition

The percentage of α-glucosidase inhibition by ethyl acetate extract varied among the eight endophytic fungi. The statistical analysis data show that the value of α-glucosidase inhibition of each sample with triplicate experiments was not significantly different (p >0.05). The data from the Tukey HSD test also show values that were not significantly different (p >0.05). The enzyme activity inhibition of the extracts (stem and flower extracts) fell between 25.64% and 29.22% (Table 1). The isolates LV-1A and LV-1C from *E. elatior* leaves exhibited the highest levels of inhibition, with inhibition values of 69.764% and 61.526%, respectively. Similar results were reported by Tiwari et al. (2017), who discovered 5 endophytic fungi from the leaves and stem of *Ficus religiosa* L., with the highest α-glucosidase inhibition value of 42±0.01%. Kaur et al. (2018) also found 17 endophytic fungi from *Azadirachta indica* A.Juss. and 15 endophytic fungi from *Bryophyllum* with the highest α-glucosidase inhibition values of 64% and 66%, respectively. As the isolates LV-1A and LV-1C showed the two highest antidiabetic potencies, they were further identified by morphological characteristics similarity and molecularly (by aligning their specific DNA sequences with those deposited in the NCBI).

Identification of the potential isolates endophytically associated with *Etlingera elatior*

The two isolates (LV-1A and LV-1C) were identified based on their colony morphology and Internal Transcribed Spacer (ITS)-based phylogeny. The degree of similarity between the ITS and 18S rDNA gene sequences obtained following alignment with those deposited in the GenBank fell from 99 to 100%. The isolates LV-1A and LV-1C were identified as *Daldinia eschscholtzii* isolate CFL 7 and *Hypoxylon trugodes* voucher YMJ 57, respectively. As shown in Table 2, *D. eschscholtzii* isolate CFL 7 had a 100% identification rate in NCBI. Meanwhile, *H. trugodes* voucher YMJ 57 had a 99.35% identification rate.

Table 2 displays the morphological characteristics of *D. eschscholtzii* isolate CFL 7 (LV-1A) and *H. trugodes* voucher YMJ 57 (LV-1C) isolates. *Daldinia* and *Hypoxylon* have a morphological characteristic of white color on the front and back sides, typical of the Xylariaceae family (e.g., well-developed stromata with several perithecia embedded, an ascal apical ring that normally turns blue in an iodine solution, and black, one-celled, or sometimes unequally two-celled ascospores with a germination slit) (Hsieh et al. 2005), initially erected based on their conspicuous stromata with internal alternating ring zones (Sir et al. 2016).

The *Hypoxylon* group is characterized by a flat ascal apical ring and a scroll-like ornamentation of the ascospore perispore in several species (Hsieh et al. 2005). Exploring the functions of endophytic fungi in lignocellulose degradation, nutrient cycling, and secondary metabolite production can lead to the production of *Hypoxylon* (U'Ren et al. 2016). As saprotrophs of wood, litter, soil, and dung, as plant pathogens in agricultural and natural systems, and as asymptomatic endophytes in the photosynthetic tissues of all lineages of land plants and lichens, these species play essential ecological functions in nature (U'Ren et al. 2016).

Based on the macroscopic characteristics on day 7 on the PDA medium, the *H. trugodes* voucher YMJ 57 isolate had a round colony shape with a white surface and bottom color, a flat elevation of the mycelium, a velvety to felty surface texture, a filamentous edge of the colony, moderate density, and a brown effect zone on the PDA medium. As a massive tissue in its colonies, the *D. eschscholtzii* isolate CFL 7 displayed the appearance of concentric zones layered with a massive sub-perithecial tissue and inconspicuous concentric zones corresponding to different hyphal orientations. On the other hand, the *H. trugodes* voucher YMJ 57 isolated concentric zones were plated on the dark side of the colony. It had a smooth surface and filamentous margin of the colony. The *D. eschscholtzii* isolate CFL 7 grew more rapidly than the *H. trugodes* voucher YMJ 57 isolates. Figure 1 displays the endophytic fungi isolates (LV1A and LV1C).

Table 2. The molecular and morphotype identification of selected endophytic fungi associated with *Etlingera elatior*

Code	Molecular analysis				Colony color		Concentric	Colony	Edge of	Texture
isolates/Species	Taxonomy ID	Score	QC (%)	% Identity	Front	Backside	circle	shape	colony	
LV-1A/*Daldinia eschscholtzii* isolate CFL 7	2004352	1033	100	100	White	White	+, special features	Circular	Filamentous	Fluffy
LV-1C/*Hypoxylon trugodes* voucher YMJ 57	326681	1237	99	99.35	White	White	+, special features	Circular	Filamentous	Velvety to felty

Molecular phylogeny and IC_{50} values of selected endophytic fungi associated with *Etlingera elatior*

The phylogenetic tree of the ITS genes from the two fungal isolates associated with *E. elatior* was generated using MEGA 7 (Kumar et al. 2016), which was constructed based on their evolutionary relationship with sequences of their counterparts deposited on the NCBI site and the neighbor-joining tree method. The phylogenetic tree constructed as shown in Figure 2 indicates that the isolates LV-1A and LV-1C share the same ancestor, but their mutations differ, making the LV-1C isolate more closely related to the *H. trugodes* species group, while LV-1A is more directly related to *D. eschscholtzii*.

The *Daldinia* sequences are monophyletic. This cladogram supports a study by Becker et al. (2020), who reported that Xylariaceae is separated into numerous main clades of *Hypoxylon* and *Daldinia* taxa, forming another distinct clade accommodated in the Hypoxylaceae. *Daldinia* and *Hypoxylon* are included in a distinct family of filamentous Ascomycota, Xylariales, containing taxa of *Daldinia* and *Hypoxylon* as a sizable monophyletic group (Hsieh et al. 2005; Becker et al. 2020).

After the screening, an IC_{50} test was performed on the two endophytic fungal isolates (*D. eschscholtzii* isolate CFL 7 and *H. trugodes* voucher YMJ 57), and the results are displayed in Table 3. The IC_{50} inhibition activity concentrations of the *D. eschscholtzii* isolate CFL 7 and *H. trugodes* voucher YMJ 57 isolates were 825 µg/mL and 738 µg/mL, respectively (Table 3).

Endophytic isolates of fungi exhibiting antidiabetic action have limitedly been reported, including the fungus *Nigrospora oryzae* from *Combretum dolichopetalum* Engl. & Diels leaves, which displayed substantial antidiabetic activity (Uzor et al. 2017). Endophytic fungi isolated from the leaves of Mango (*Mangifera indica* L.) and Jambolan (*Syzygium cumini* L.) inhibited amylase significantly at 49-59% and 62%, respectively (Khan et al. 2019). Endophytic fungi from *Cassia siamea* Lamk. leaves have IC_{50} values of 28.40-262.46 ppm as α-glucosidase inhibitors (Mun'im et al. 2013). Endophytic actinomycetes isolated from *Catharanthus roseus* (L.) G.Don had significant α-glucosidase inhibitory activity in butanol fraction (72.16±1.0%) and ethyl acetate fraction (70.76±0.49%) (Jasmine and Agastian 2013). *Salvadora oleoides* Decne. associated fungal endophytes demonstrated decreases in blood glucose of 11.3% to 28.04% compared to the conventional medicine tolbutamide (40%) (Dhankhar et al. 2013). Endophytic fungi from mahogany (*Swietenia macrophylla* G. King) seed ethyl acetate extracts revealed IC_{50} values of 73.64 µg/mL (Ramdanis et al. 2012). The endophytic fungus *Ocimum sanctum* had IC_{50} values of 29.51 µg/mL and 31.26 µg/mL to inhibit the α-glucosidase enzyme (Kumar et al. 2014).

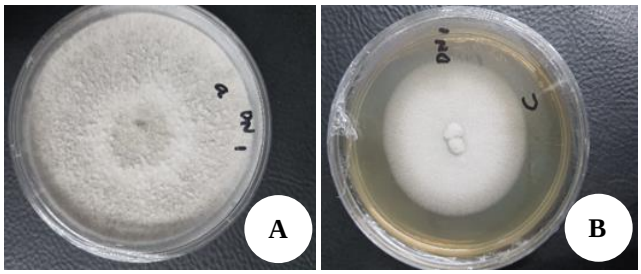

Figure 1. Morphology of endophytic fungi: A. *Daldinia eschscholtzii* isolate CFL7, LV-1A, B. *Hypoxylon trugodes* voucher YMJ 57, LV-1C

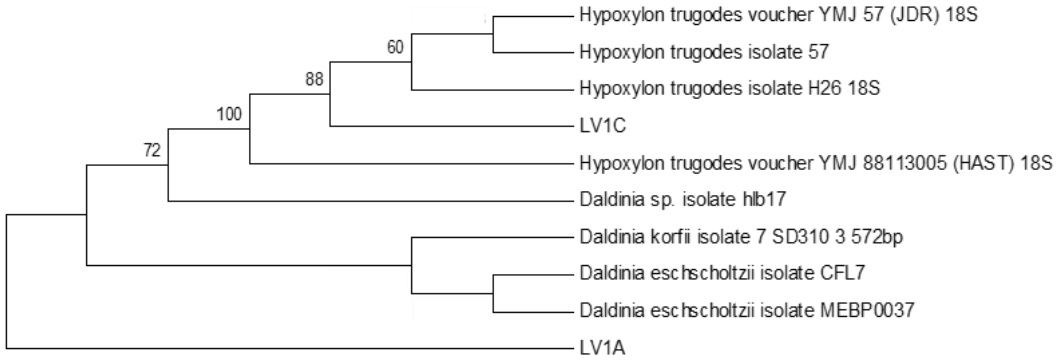

Figure 2. A phylogenetic tree of selected endophytic fungi from *Etlingera elatior*: A. LV-1A and B. LV-1C based on ITS region sequences of Neighbor-Joining (Unrooted Tree) by Mega 7

Table 3. The IC_{50} of α-glucosidase inhibition of endophytic fungi associated with a plant belonging to the family of Zingiberaceae

Endophytic fungi	Sources of plant	Organ plant	IC_{50} (µg/mL)	Ref
Daldinia eschscholtzii isolate CFL 7	*Etlingera elatior*	Leaf	825	*This study*
Hypoxylon trugodes voucher YMJ 57	*Etlingera elatior*	Leaf	738	*This study*
Isolate Bo.Ci.Cl.R1-R5	Turmeric	Rhizome	336.22	Septiana et al. 2019

Our study demonstrates that it is possible to obtain antidiabetic endophytic fungi from *E. elatior* leaves with α-glucosidase inhibitory activity of 61.53-69.76% (Table 1). However, we didn't detect antioxidant compound in the secondary metabolites used as sample extracts. Therefore, additional or further research must be conducted on the metabolomics analysis of these compounds' constituents.

Turmeric and *E. elatior* are members of the Zingiberaceae family. With an IC_{50} value of 336.22 µg/mL, the ethyl acetate extract of endophytic fungal isolates from turmeric rhizomes was the most effective in inhibiting α-glucosidase enzyme activity (Septiana et al. 2019). In our study, the *Daldinia* and *Hypoxylon* isolates from the *E. elatior* leaf extracts inhibited the α-glucosidase enzyme activity less effectively than the extract of endophytic fungal isolates from turmeric rhizomes. However, the modest antidiabetic bioactivity observed requires an extensive evaluation of its applicability in other assays. Wagenaar et al. (2000) reported that *Hypoxylon* produces cytochalasins, which share antitumor properties with the alkaloids commonly found in endophytic fungi. Recommendations for further research on *Hypoxylon* should be founded on anticancer studies.

In addition, this is the first (novel) endophytic report of the genera *Daldinia* and *Hypoxylone* in *E. elatior*, providing the first molecular and morphological evidence for monocotyledons in Indonesia. Bioactive volatiles from an endophytic *Daldinia cf.* concentric isolate inhibits the viability of the parasitic plant nematode *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Liarzi et al. 2016). Therefore, isolating *Daldinia* could be considered a novel alternative biocontrol method for plants. The presence of two selected endophytic isolates from *E. elatior* is fascinating to discuss, and further research is required on tropical species of *Daldinia* to complete the polyphasic taxonomy of Xylyriacecea, for which relatively few recently collected specimens are available. The presence of the secondary metabolite distinguishes *Daldinia* daldinone A in its stromata (Hellwig et al. 2005). It is strongly suggested that these compounds must be studied to complete the chemotaxonomic marker characteristics for these isolates.

Overall, it can be concluded that endophytic fungi could be isolated and screened from the roots, leaves, flowers, and stems of *E. elatior*. Only 8 of 29 isolates showed antidiabetic activity, two of which showed the best inhibitory activity against enzymatic activity (LV-1A and LV-1C with optimal inhibition concentrations of 69.764%±0.308% and 61.526±0.399%, respectively). Molecular analysis revealed that LV-IA and LV-1C are closely related to *Daldinia eschscholtzii* isolate CFL 7 and *Hypoxylon trugodes* voucher YMJ 57. The IC_{50} values of LV-IA and LV-1C were 825 µg/mL and 738 µg/mL, respectively. Therefore, they could be considered potential isolates in preventing or treating diabetes mellitus.

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