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ORIGINAL ARTICLE

α-Glucosidase inhibitor activity of endophytic fungi isolated from oil palm leaves (*Elaeis guineensis* Jacq)

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ABSTRACT

Indonesia ranks seventh globally in diabetes prevalence, with an estimated rate of 4.8%, and more than half of the cases remain undiagnosed. By 2030, the number of individuals with diabetes in Indonesia is projected to reach 21.3 million. Alpha-glucosidase inhibitors are commonly used to prevent the progression of prediabetes; however, their use is often associated with adverse side effects. Therefore, research is needed to identify natural sources of alpha-glucosidase inhibitors.

This study aimed to evaluate the in vitro alpha-glucosidase inhibitory activity of endophytic fungi isolated from oil palm leaves. The research consisted of fungal isolation, microscopic characterization, and analysis of α-glucosidase inhibitory activity. Two pure endophytic fungal isolates, designated JES1 and JES2, were successfully obtained. Isolate JES1 exhibited a denser mycelial network with well-defined septation, whereas JES2 displayed loosely arranged, curved hyphae, suggesting that the variation was likely due to differences in growth phase or sampling location rather than taxonomic divergence. Microscopic observations identified JES1 and JES2 as *Colletotrichum* sp. and *Fusarium* sp., respectively.

The α-glucosidase inhibitory assay showed that isolates JES1 and JES2 demonstrated inhibitory activities of 79.4% and 20.8%, respectively, while the positive control, acarbose, exhibited an activity of 81%. These results indicate that endophytic fungi isolated from oil palm leaves have potential as natural sources of bioactive compounds with α-glucosidase inhibitory properties.

Keywords: endophytic fungi, oil palm leaves, α-glucosidase inhibitor

INTRODUCTION

The prevalence of diabetes in Indonesia ranks seventh globally, following China, India, the United States, Brazil, Russia, and Mexico. The national prevalence of diabetes mellitus (DM) is approximately 4.8%, and more than half of these cases (58.8%) remain undiagnosed. By 2030, the number of people living with diabetes in Indonesia is projected to reach 21.3 million. Diabetes continues to pose a serious public health concern, with the number of affected individuals rising steadily each year due to population growth, aging, unhealthy lifestyles, poor dietary habits, and obesity.[1]

α-Glucosidase is an enzyme located in the brush border membrane that catalyzes the hydrolysis of carbohydrates. Inhibiting its activity has been shown to reduce glucose absorption and lower postprandial blood glucose levels. Therefore, α-glucosidase inhibitors represent an important therapeutic strategy for diabetes management, with acarbose widely used as a potent inhibitor in diabetic patients.[2] The primary side effects of acarbose are gastrointestinal, including abdominal distension, diarrhea, and potential hypoglycemic symptoms. Islam et al.[3] reported that gastrointestinal side effects occur more frequently with other medications.

A previous study demonstrated that an eight-week intervention with palm leaf (*Elaeis guineensis*) extract in healthy adults with prediabetes (aged 21–65 years; BMI ≥25 and <40 kg/m²) resulted in reductions in fasting plasma glucose and insulin levels, glucose and insulin area under the curve, and insulin resistance, while improving insulin sensitivity. A dose of 500 mg *E. guineensis* extract produced more consistent glycemic control than a 1000 mg dose.[4] These findings suggest that palm leaves have potential as an antidiabetic agent.

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However, one major challenge lies in the extraction of bioactive compounds from palm leaves, as this process requires direct access to palm trees and involves considerable financial resources. A potential alternative is the use of endophytic microbes associated with palm leaves. The bioactive compounds present in medicinal plants are often linked to the presence of endophytic microorganisms inhabiting their tissues. Endophytic fungi, in particular, are known for producing metabolites with diverse biological activities, including α-glucosidase inhibition. Several studies have reported the isolation of endophytic fungi capable of producing α-glucosidase inhibitory compounds, such as Xylariaceae sp. from *Quercus gilva*, Pseudocercospora sp. from *Elaeocarpus sylvestris*, and *Penicillium pimiteouiense* from *Simarouba glauca*.[5]

To date, there have been no reports on α-glucosidase inhibitory activity from endophytic fungi isolated from oil palm leaves. Therefore, this study aimed to investigate the potential of endophytic fungi derived from oil palm (*E. guineensis*) leaves to produce α-glucosidase inhibitory compounds as potential candidates for future antidiabetic drug development.

METHOD

Palm leaf sample confirmation test

Palm leaves were washed thoroughly with running water prior to sterilization. The leaves were then surface-sterilized by sequential immersion in 70% ethanol for 3 minutes, 5.25% sodium hypochlorite (NaOCl) solution for 5 minutes, and 70% ethanol for 30 seconds. The sterilized leaves were subsequently rinsed six times with sterile physiological saline (0.85% NaCl) and air-dried on sterile tissue paper inside a biological safety cabinet (BSC). To verify the effectiveness of the sterilization process, an aliquot of the final rinse solution was cultured on Nutrient Agar (NA) in Petri dishes and incubated at 30°C for 5 days. The procedure was continued only if no microbial growth was observed on the NA surface.[6]

Isolation of endophytic fungi

Endophytic fungi were isolated using Potato Dextrose Agar (PDA) medium. Sterile leaf disks, 6 mm in diameter, were prepared using a cork borer and placed on PDA medium supplemented with 0.1% chloramphenicol to inhibit bacterial growth. The cultures were incubated at 30°C for 7 days. Emerging fungal colonies were differentiated based on morphology, size, and color. Representative isolates were subcultured twice on fresh PDA medium and stored at 4°C for further analysis.[7]

Examination of endophytic fungal colonies

Fungal identification was performed based on macroscopic and microscopic characteristics. Macroscopic observations included colony morphology, surface color, and reverse coloration. Microscopic features were examined by staining with lactophenol blue and observing under a compound microscope.[8]

Fermentation of endophytic fungi

Fermentation was carried out in Potato Dextrose Broth (PDB) medium. Seven-day-old mycelial cultures grown on PDA were excised into five 6 mm discs using a sterile cork borer and inoculated into 10 mL of PDB. The cultures were incubated in a shaking incubator at 30°C and 150 rpm for 21 days. The fermented broth was centrifuged at 3000 rpm for 20 minutes, and the resulting supernatant was filtered through Whatman No. 1 filter paper.[9]

α-Glucosidase inhibitory activity assay

The α-glucosidase inhibitory activity was evaluated using the method described by Mugaranja and Kulal[10] with slight modifications. Briefly, 50 µL of the enzyme mixture containing 40 µL of α-glucosidase (1 U/mL) and 10 µL of phosphate buffer (pH 7) was added to each microplate well. The enzyme solution (1 U/mL) was prepared by dissolving 2.695 mg of α-glucosidase (Sigma-Aldrich, G5003) in 50 mL of phosphate buffer (pH 7). The reaction mixtures were incubated at 37°C for 20 minutes, followed by the addition of 50 µL of 20 mM p-nitrophenyl-α-D-glucopyranoside (PNPG) substrate. The mixtures were incubated again at 37°C for 30 minutes. The reaction was terminated by adding 100 µL of 200 mM sodium carbonate (Na_2CO_3) solution. Reaction blanks were prepared by replacing samples or enzymes with buffer, as appropriate. Controls contained enzyme and buffer without the test sample. For each sample, a corresponding blank containing buffer instead of enzyme was also prepared. The absorbance of p-nitrophenol released was measured at 405 nm using a microplate reader. Acarbose (10 mg/mL) was used as a positive control.[10] The detailed experimental setup is shown in Table 1.

Table 1. Design of α-Glucosidase Inhibitor Enzymatic Reaction

Mixture	Cuvette A0 (µL)	Cuvette AI (µL)	Cuvette AI0 (µL)	Cuvette AI1 (µL)
Sample	-	-	50	50
Enzyme	-	40	-	40
Buffer (pH 7)	100	60	50	10
Incubation at 37°C for 20 minutes				
PNPG	50	50	50	50
Incubation at 37°C for 20 minutes				
Na_2CO_3	100	100	100	100

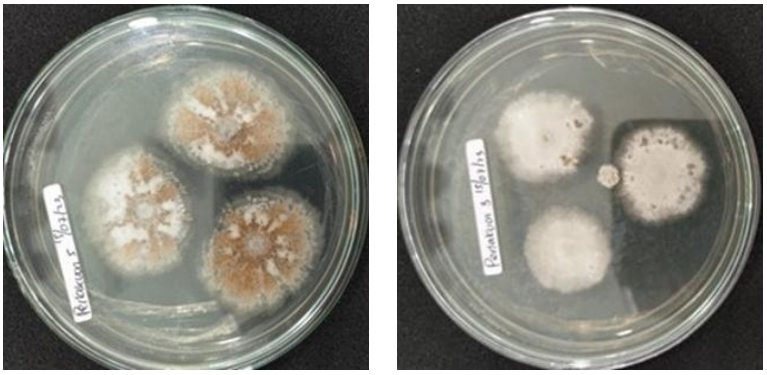
The inhibitory activity of isolates and acarbose against the α-glucosidase enzyme was calculated using the following formula:

% inhibition = (absorbance A1-A0)-(absorbance AI1-absorbance AI0)/(absorbance A1-absorbance A0) x 100%[11]

RESULTS

Isolation of endophytic fungi from oil palm leaves

In this study, endophytic fungi were successfully isolated from oil palm leaves. After purification, the isolates were determined to consist of two distinct fungal strains. The preliminary results of fungal isolation prior to purification are presented in Figure 1.



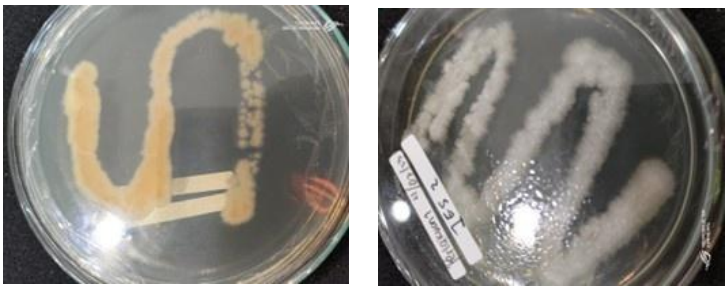
(a) (b)

Figure 1. Isolation results of endophytic fungi from oil palm leaves after seven days of incubation at 30°C on PDA medium.

Two different colony morphologies were observed in Figure 1. In Figure 1(a), the colony displayed both white and orange colors, indicating that it was not yet pure. In contrast, Figure 1(b) shows a single-colored colony with a cotton-like surface, suggesting a purified isolate. The colony in Figure 1(a) was round and compact, with a brownish-orange center, white margins, and a distinct concentric radial pattern. The texture was moderately compact and not overly cottony, indicating a dense mycelial structure. Figure 1(b) shows colonies that were white to grayish-white, circular, and radially patterned, with a cottony-floccose center and relatively smooth edges.

Macroscopic and microscopic examination of endophytic fungal colonies

Both isolates were then purified, and the results are shown in Figure 2. Figure 2(a) shows colonies with a cream-yellow to orange appearance and spreading growth along the inoculation streak. The texture was smooth to slightly slimy. This isolate was designated as JES1. Figure 2(b) shows bright white colonies with a cottony texture, compact mycelium, and growth along the inoculation streak, but without a slimy surface. This isolate was designated as JES2.



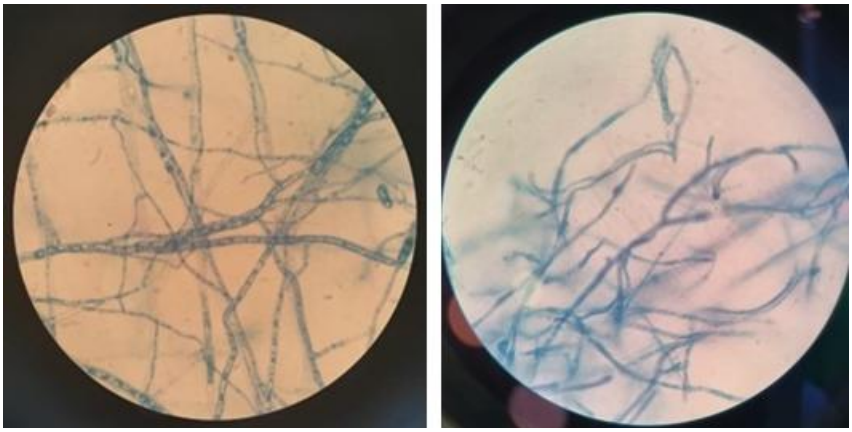
(a) (b)

Figure 2. Purified isolates of endophytic fungi on PDA medium after seven days of incubation at 30°C. Isolates in Figures 2(a) and 2(b) were designated as JES1 and JES2, respectively.

Microscopic observation using lactophenol blue staining was then conducted, and the results are shown in Figure 3.

Microscopic examination of isolate JES1 (Figure 3(a)) revealed hyaline, septate hyphae that were long, slender, and branched, with clearly defined septa and consistent spacing. The hyphae exhibited simple branching with variable angles and occasional curvature. Lactophenol cotton blue staining colored the hyphae purple-blue, clearly outlining the cell walls. No reproductive structures such as conidiophores, vesicles, sporangia, or conidial heads were observed. Conidia were also absent, indicating that isolate JES1 was in the vegetative growth phase. These morphological characteristics are consistent with endophytic Ascomycota, particularly *Colletotrichum* species.

Microscopic observation of isolate JES2 revealed curved to slightly wavy septate hyphae with uniform spacing. Some hyphal tips showed slight thickening; however, no distinct reproductive structures such as conidiophores, sporangia, or conidial heads were observed. The absence of free conidia or sporulation structures indicates that the isolate was also in an active vegetative growth phase. These features suggest that isolate JES2 shares morphological similarities with *Fusarium* species.



(a) (b)

Figure 3. Microscopic observation of JES1 (a) and JES2 (b) Isolate under 100x magnification

Alpha-Glucosidase inhibitory activity

Before the α-glucosidase inhibition assay, both endophytic fungal isolates were fermented in PDB medium to obtain the culture supernatant. The supernatant was subsequently used to assess inhibitory activity. The absorbance values of JES1, JES2, and acarbose (as a positive control) are presented in Table 2. Based on the calculated results, the α-glucosidase inhibitory activity was 79.4% for JES1, 20.8% for JES2, and 81% for acarbose.

Table 2. Absorbance measurements of fungal supernatants and acarbose

Supernatant	A0	A1	A10	A11
JES1	0	0.107	0.094	0.116
JES2	0	0.048	0.075	0.113
Acarbose	0	0.080	0.049	0.064

DISCUSSION

Endophytic fungi are vital microbial "bioactive compound factories" that synthesize a wide array of medically and agriculturally important metabolites without inducing plant disease. They produce bioactive compounds across multiple structural classes, including alkaloids, peptides, steroids, terpenoids, and flavonoids.[12] These compounds exhibit diverse therapeutic activities, such as antibacterial, antiviral, antifungal, anticancer, and immunosuppressive properties.[13] Endophytic fungi also possess strong potential for producing antidiabetic compounds, particularly α-glucosidase inhibitors. Numerous studies have provided compelling evidence of this capability, with specific species such as *Penicillium brefeldianum* and *Aspergillus* sp. identified as promising sources of such metabolites.[14]

In this study, the JES1 isolate, identified as *Colletotrichum*, exhibited relatively high α-glucosidase inhibitory activity of 79.4%, while JES2 showed 20.8% activity. The inhibitory activity of JES1 was comparable to that of acarbose (81%), despite being obtained from an unpurified fermentation supernatant, indicating its strong potential as a natural α-glucosidase inhibitor. The pronounced difference in α-glucosidase inhibitory activity between JES1 and JES2 may result from variations in secondary metabolite production, which can be influenced by fungal genus, growth phase, and metabolic regulation. According to K. Wadhwa et al. (2024), fungi produce secondary metabolites at different growth stages, with production patterns varying across genera.[15]

Colletotrichum is a morphologically and ecologically diverse fungal genus that includes endophytes, pathogens, and saprobes of considerable biological and research importance. A comprehensive study by F. Liu et al. (2022) reported that the genus comprises 280 species supported by molecular data, including 30 newly described species. Notably, 33 *Colletotrichum* species have been exclusively identified as endophytes. Among these, *C. siamense*, *C. karsti*, *C. fructicola*, and *C. gloeosporioides* are the most frequently detected, exhibiting broad host ranges.[16]

Endophytic *Colletotrichum* species produce various compounds with potential antidiabetic activity, although current evidence remains preliminary. Sen-feng Sun et al. (2021) identified a compound from *Colletotrichum gloeosporioides* with potent PTP1B inhibitory activity (IC_{50} = 0.84 μM), suggesting significant antidiabetic potential.[17] Additionally, Mohamed M. M. Abdel Razek et al. (2023) demonstrated through in silico analysis that Colletotrichalactone A could inhibit α-amylase and the human sodium-glucose cotransporter 2, reinforcing the genus's relevance in antidiabetic research.[18]

Endophytic fungi inhibit α-glucosidase enzymes by producing structurally diverse secondary metabolites that interact with and block the enzyme's active site, thereby preventing glucose hydrolysis. Several studies have documented potent inhibition through distinct chemical classes. For instance, Yingnan Wu et al. (2018) reported diphenyl ethers and phenolic sesquiterpenoids exhibiting IC_{50} values as low as 1.5–4.5 μM.[19] To the best of our knowledge, this study is the first to report α-glucosidase inhibitory activity from endophytic fungi isolated from oil palm (*Elaeis guineensis*) leaves. However, this work is limited by reliance on morphological identification without molecular confirmation and by the absence of IC_{50} determination, which should be addressed in future research.

CONCLUSION

This study demonstrated that endophytic fungi isolated from oil palm (*Elaeis guineensis*) leaves exhibit significant α-glucosidase inhibitory potential. Two isolates, designated JES1 and JES2, were successfully obtained and morphologically identified as *Colletotrichum* sp. and *Fusarium* sp., respectively. The α-glucosidase inhibition assay showed that isolate JES1 displayed strong inhibitory activity (79.4%), comparable to the commercial antidiabetic drug acarbose (81%), whereas JES2 exhibited substantially lower inhibition (20.8%). These results suggest that endophytic fungi associated with oil palm leaves may serve as promising and underexplored sources of natural α-glucosidase inhibitors. Further research is necessary to purify and characterize the active compounds, determine their IC_{50} values, confirm fungal identities through molecular analysis, and assess their safety and efficacy in vivo. Overall, this study provides a scientific basis for the potential development of endophytic fungi-derived antidiabetic agents.

REFERENCES

1. Nasution F, Andilala A, Siregar AA. Faktor risiko kejadian diabetes mellitus. J Ilmu Kesehat [Internet]. 2021 May 20;9(2):94. Available from: https://ejurnaladhkdr.com/index.php/jik/article/view/304
2. Chen P, Zhang Q, Dang H, Liu X, Tian F, Zhao J, et al. Screening for potential new probiotic based on probiotic properties and α-glucosidase inhibitory activity. Food Control. 2014 Jan;35(1):65–72.
3. Islam T, Akter A, Laila R, Renu R, Khatun S, Ishrat S. Acarbose is as effective as metformin in reducing central obesity in infertile women with polycystic ovary syndrome. Int J Reprod Contraception, Obstet Gynecol. 2024 Sep 26;13(10):2741–7.
4. Kalman DS, Schwartz HI, Feldman S, Krieger DR. Efficacy and safety of Elaeis guineensis and Ficus deltoidea leaf extracts in adults with pre-diabetes. Nutr J. 2013 Dec 1;12(1):36.
5. Septiana E, Rachman F, Hapsari Y, Yadi Y, Bustanussalam B, Rahmawati SI, et al. The potential of Endophytic Fungal Extract Isolated from Cinnamon (Cinnamomum burmannii) as Antidiabetic and Antioxidant. J Kim Sains dan Apl. 2019 Nov 30;22(6):275–82.
6. Nxumalo CI, Ngidi LS, Shandu JSE, Maliehe TS. Isolation of endophytic bacteria from the leaves of Anredera cordifolia CIX1 for metabolites and their biological activities. BMC Complement Med Ther. 2020 Dec 7;20(1):300.
7. Indrianingsih AW, Tachibana S. α-Glucosidase inhibitor produced by an endophytic fungus, Xylariaceae sp. QGS 01 from Quercus gilva Blume. Food Sci Hum Wellness. 2017 Jun;6(2):88–95.
8. Ramdanis R, Soemiati A, Mun'im A. Isolation of α-glucosidase inhibitory activity of fungi from mahogany(Swietenia macrophylla King) seeds. Int J Med Aromat Plants. 2012;2(3):447–52.
9. Octaviani M, Ameliah WY, Frimayanti N, Djohari M, Fadhli H. Isolation of Endophytic Fungus from Leaves of Uncaria cordata (Lour.) Merr and Antibacterial Activity Against Propionibacterium acnes and Escherichia coli. Borneo J Pharm. 2022 Aug 31;5(3):279–87.
10. Mugaranja KP, Kulal A. Alpha glucosidase inhibition activity of phenolic fraction from Simarouba glauca: An in-vitro, in-silico and kinetic study. Heliyon. 2020 Jul;6(7):e04392.
11. Susilowati A, Dewi CPY, Sari SLA. Isolation and identification of endophytic bacteria from Salak Pondoh (Salacca edulis) fruit as a-glycosidase inhibitor producer. Biosaintifika. 2019;11(3):352–9.
12. Manganyi MC, Ateba CN. Untapped Potentials of Endophytic Fungi: A Review of Novel Bioactive Compounds with Biological Applications. Microorganisms. 2020 Dec 6;8(12):1934.
13. Wijesekara T, Xu B. Health-Promoting Effects of Bioactive Compounds from Plant Endophytic Fungi. J Fungi. 2023 Oct 8;9(10):997.
14. Bai Y, Yi P, Zhang S, Hu J, Pan H. Novel Antioxidants and α-Glycosidase and Protein Tyrosine Phosphatase 1B Inhibitors from an Endophytic Fungus Penicillium brefeldianum F4a. J Fungi. 2021 Oct 27;7(11):913.
15. Wadhwa K, Kapoor N, Kaur H, Abu-Seer EA, Tariq M, Siddiqui S, et al. A Comprehensive Review of the Diversity of Fungal Secondary Metabolites and Their Emerging Applications in Healthcare and Environment. Mycobiology. 2024 Nov 3;52(6):335–87.
16. Liu F, Ma ZY, Hou LW, Diao YZ, Wu WP, Damm U, et al. Updating species diversity of Colletotrichum , with a phylogenomic overview. Stud Mycol. 2022 Mar 1;101(1):1–56.
17. Sun SF, Zhu S, Cao HY, Liu YB, Yu SS. Tridepsides from the endophytic fungus colletotrichum gloeosporioides associated with a toxic medicinal plant tylophora ovata. Frigid Zo Med. 2021 Sep 1;1(1):45–51.

18. AbdelRazek MMM, Elissawy AM, Mostafa NM, Moussa AY, Elanany MA, Elshanawany MA, et al. Chemical and Biological Review of Endophytic Fungi Associated with Morus sp. (Moraceae) and In Silico Study of Their Antidiabetic Potential. Molecules. 2023 Feb 10;28(4):1718.
19. Wu Y, Chen Y, Huang X, Pan Y, Liu Z, Yan T, et al. α-Glucosidase Inhibitors: Diphenyl Ethers and Phenolic Bisabolane Sesquiterpenoids from the Mangrove Endophytic Fungus Aspergillus flavus QQSG-3. Mar Drugs. 2018 Sep 1;16(9):307.