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Research Paper

Antidiabetic Activity of Endophytic Fungi Extract from Leaves of Kembang bulan (*Tithonia diversifolia* (Hemsley) A. Gray) Through α-Amylase Enzyme Inhibition

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Abstract
Diabetes mellitus is a serious health problem with an increasing number of sufferers. In this context, an important method for controlling blood sugar levels is the derivation of α-amylase enzyme inhibitors from natural materials such as endophytic fungi. Therefore, this research aimed to analyze the potential of endophytic fungi from *Tithonia diversifolia* leaves as α-amylase enzyme inhibitors, secondary metabolites, as well as the character and identity of potential endophytic fungi. The stages included isolation and identification of endophytic fungi, extraction of secondary metabolites, α-amylase inhibition tests, analysis of bioactive compounds using Thin Layer Chromatography (TLC), and identification of potential endophytic fungi. The results showed that DT3J1 (67.83 μg/mL), DM3J1 (69.36 μg/mL), DT2J2 (80.42 μg/mL), and DT4J1 (88.64 μg/mL) of the eight endophytic fungi isolates reported high potential as inhibitors of the α-amylase enzyme, with IC_{50} values classified as strong. Potential fungal extracts contain bioactive compounds such as alkaloids, flavonoids, phenols, and terpenoids. Meanwhile, molecular identification showed that potential endophytic fungi consisted of *Curvularia pseudointermedia*, *Diaporthe passifloricola*, *Nodulisporium verrucosum*, and *Muyocopron laterale* species. This research also provided scientific evidence on the potential of the endophytic fungus *Tithonia diversifolia* as a candidate for antidiabetic drugs.

Keywords
Diabetes Mellitus, α-Amylase Inhibitors, Endophytic Fungi, *Tithonia diversifolia*, Bioactive Compounds, IC_{50}

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1. INTRODUCTION

Diabetes mellitus is a hyperglycemic disease caused by the inability of the body to convert glucose into energy due to endocrine system disorders characterized by metabolic disorders in proteins, lipids, and carbohydrates (Antar et al., 2023). This disease is among the main health problems in Indonesia, which is characterized by the increasing number of sufferers every year. The α-amylase found in saliva and pancreatic fluid is a trigger for increased blood glucose levels in diabetics because the enzyme breaks down carbohydrates into glucose (Peyrot des Gachons and Breslin, 2016). Therefore, inhibition of α-amylase activity is needed to control blood glucose levels. Acarbose, metformin, and voglibose are medications used to control blood glucose levels through the inhibition of digestive enzymes. However, several studies have shown that the use of these drugs can result in side effects such as gastrointestinal disturbances, dizziness, nausea, and vomiting (Thangwaritorn et al., 2024).

Based on the description above, natural α-amylase enzyme inhibitors reduce side effects and increase sources of raw drug materials. In this context, *Tithonia diversifolia* acts as enzyme inhibitor with an efficacy of controlling blood glucose levels (Almayda et al., 2024; Suherman et al., 2022). The leaves of the plant contain alkaloids, saponins, glycosides, tannins, and essential oils for inhibiting the activity of the α-amylase enzyme (Akinwunmi et al., 2017; Muniroh and Solfaine, 2022; Tamfu et al., 2022). Endophytic fungi can inhibit α-amylase enzyme and produce bioactive compounds (Hashem et al., 2023). In this context, endophytic fungi produce metabolite compounds in a shorter time compared to cultivating the host plants (Gupta et al., 2023). Several research were carried out relating to the potential of endophytic fungal extracts in Indonesia. Therefore, this research analyzes the potential of endophytic fungal extracts from *Tithonia diversifolia* leaves in inhibiting α-amylase enzyme. The development of endophytic fungi as raw materials for diabetes therapy drugs is supported by secondary metabolite content of fungal extracts and identity.

2. EXPERIMENTAL SECTION

2.1 Materials

In this research, the materials used are *Tithonia diversifolia* leaves including young and old leaves, sterile distilled water, 70% alcohol, ethyl acetate solvent, Potato Dextrose Agar (PDA), potatoes, dextrose, sodium hypochlorite (NaOCl) 3%, dimethyl sulfoxide (DMSO), dinitrosalicylic acid (DNS), phosphate buffer pH 6.9, acarbose (Kimia Farma), α-amylase enzyme from human saliva (Sigma-Aldrich), F 254 silica gel plates, and the Plant Genomic Tiangen Kit.

2.2 Instrumentation and Characterization

α-Amylase enzyme inhibition test used spectrophotometer UV-Vis (Shimadzu UV-1240) to check the absorbation of control and test solution. Visualization of spots on TLC plate used UV transluminator (CAMAG UV Cabinet 4). Microscopic observation of endophytic fungal morphology using a trinoculer microscope (Meiji Techno type MT4300L, HD 1500T).

2.3 Method

2.3.1 Isolation and Purification

Endophytic fungi were isolated from the leaves of *Tithonia diversifolia* which identified from plant biosystematics laboratory at Sriwijaya University under collection number 349/UN9.1.7/4/EP/2024. The samples were immersed in 70% alcohol for 30 seconds to conduct surface sterilization before adding 3% sodium hypochlorite (NaOCl). After 1 minute, the samples were rinsed with sterile water, and dried. The leaves were cut into 1×1 cm pieces, planted in a Petri dish containing Potato Dextrose Agar (PDA) and incubated at 25-27°C until endophytic fungi grew. The colonies were transferred with different morphologies into a PDA medium and incubated (Widjajanti et al., 2021).

2.3.2 Cultivation and Extraction

A cork borer was used to inoculate the isolate with 10 agar plugs at 0.5 cm and placed into 500 mL of PDB in a 1 liter bottle. Additionally, the culture was incubated for 35 days and the mycelium was separated using filter paper. Extraction was carried out twice by adding ethyl acetate solvent to PDB in a 1:1 ratio and concentrated using a rotary evaporator at 40°C to obtain a thick extract (Oktiansyah et al., 2024).

2.3.3 In Vitro α-Amylase Enzyme Inhibition Test with Endophytic Fungal Extract

The antidiabetic activity was determined using an in vitro α-amylase enzyme inhibition test (Modification of Elya et al. (2015); Siregar et al. (2022)). 500 μL α-amylase enzyme solution was pipetted and mixed with 500 μL of fungal extract. The mixture was incubated at 37°C for 10 minutes and 1000 μL of 1% starch solution was added as a substrate before incubating for 3 minutes. Additionally, DNS reagent (1000 μL) was added to the solution and incubated in boiling water for 10 minutes. Negative controls were performed using the same procedure but replacing the fungal extract sample with 500 μL of phosphate buffer solution. Blank control and samples were also made by replacing the enzyme solution with a phosphate buffer. Acarbose was used as a positive control and treated as the fungal extract sample. The absorbance values of the control and samples were measured at a wavelength of 540 nm using a spectrophotometer. The α-amylase inhibition test was conducted at 31.25 ppm, 62.5 ppm, 125 ppm, 250 ppm, and 500 ppm. The percentage of the enzyme inhibition was calculated using the following Equation (1).

$$\%\text{Inhibition} = \frac{(\text{Control Abs} - \text{Sample Abs})}{\text{Control Abs}} \times 100\% \quad (1)$$

Note :

Control Abs =Abs of negative control solution-Abs of negative control blank solution

*S*ample Abs =Abs of sample solution-Abs of sample blank solution

The inhibition test data, including the average absorbance and percentage, were analyzed using Microsoft Excel. Meanwhile, the IC_{50} value was calculated using the linear regression equation $y = a + bx$, where the variables x and y are the sample concentration and the percentage inhibition, respectively. a is the gradient value in the linear regression equation and b is the constant value in the linear regression equation. The IC_{50} value is determined from the log value of x when y = 50 or calculated using the Equation (2).

$$IC_{50} = \frac{50 - a}{b} \quad (2)$$

2.3.4 Thin Layer Chromatography (TLC) of Secondary Metabolite Compounds from Potential Endophytic Fungal Extracts

Selected endophytic fungal extracts of 5 mg were prepared and dissolved in 1 mL of the solvent used for extraction. The stationary phase used was a silica gel F 254 plate with dimensions of 1 cm×5 cm, while the mobile phase was a mixture of 10 mL specific solvents in a chamber. The solution was pipetted using a capillary pipette and spotted on an activated silica F 254 Thin Layer Chromatography (TLC) plate. In addition, the plate was eluted with a mixture of n-hexane and ethyl acetate before observing the spots under UV light at 254 nm and 366 nm. Compound identification was also carried out by comparing the Rf value and color of the spots on the TLC plate after being sprayed with 10% H_2SO_4 reagent and heated on a hot plate (Nasution et al., 2024). In this context, the Rf value is calculated using the following Equation 3.

$$\text{Rf} = \frac{\text{Distance traveled by solute (cm)}}{\text{Distance traveled by solvent (cm)}} \quad (3)$$

Table 1. Macroscopic Structure of Endophytic Fungus Discovered in *Tithonia diversifolia* Leaves

Isolate	Surface Colony	Reverse Colony	Structure	Elevation	Pattern
DT1J1	Dark Green	Dark Green	Cottony	Umbonate	Zonate
DT2J1	Yellowish cream	Orange	Glabrous	Verrugose	Zonate
DT2J2	Yellowish Green	Dark Green	Cottony	Umbonate	Spread
DT3J1	White	White	Flocculent	Rugose	Radiate
DT4J1	Brownish Gray	Brown	Velvety	Flat	Radiate
DM1J1	White	White	Cottony	Umbonate	Zonate
DM3J1	Brownish Gray	Yellowish Briwn	Velvety	Flat	Zonate
DM4J1	Dark Green	Dark Green	Cottony	Umbonate	Zonate

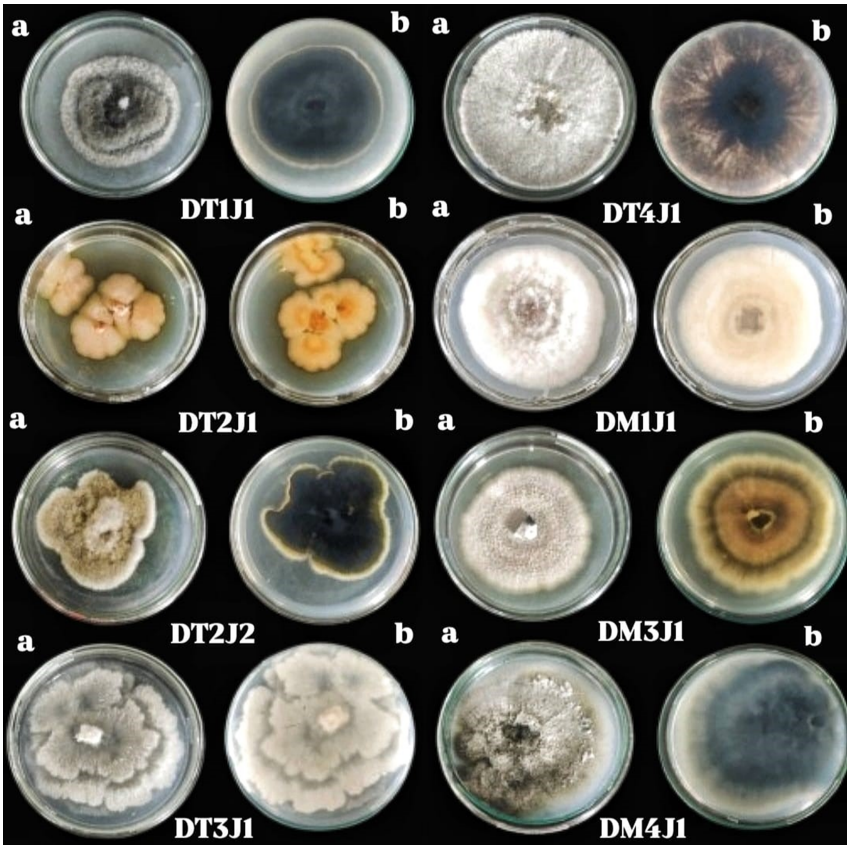

Figure 1. Macroscopic Characteristics of Endophytic Fungus Discovered in *Tithonia diversifolia* Leaves (a. Front View; b. Reverse View)

2.3.5 Identification of Potential Endophytic Fungal Isolates
Based on phenotypic and molecular characterization, identification was carried out on endophytic fungal isolates with a high potential for inhibiting the α-amylase enzyme. The macroscopic characteristics observed included colony color, inverted colony color, colony texture, growth direction, and growth rate. Meanwhile, the microscopic characteristics comprised hyphae type (septate or non-septate), hyphae branching, hyphae pigmentation (hyaline or dark), spore shape, and spore color (Nurnawati et al., 2021).

Molecular analysis started with the DNA isolation stage carried out following kit protocol. Meanwhile, DNA amplification was conducted in the ITS (Internal Transcribed Spacer) region using the ITS1 and ITS4 primer pairs. The ITS1 primer (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used as the forward and reverse primers, respectively. DNA samples were prepared for sequencing and sent to Macrogen Korea. The sequences from the primers were compiled by adopting GeneStudio software and identified at the species level using similarity analysis with the Basic Local Alignment Search Tool (BLAST) GenBank (https://blast.ncbi.nlm.nih.gov/blast.cgi). Fur-

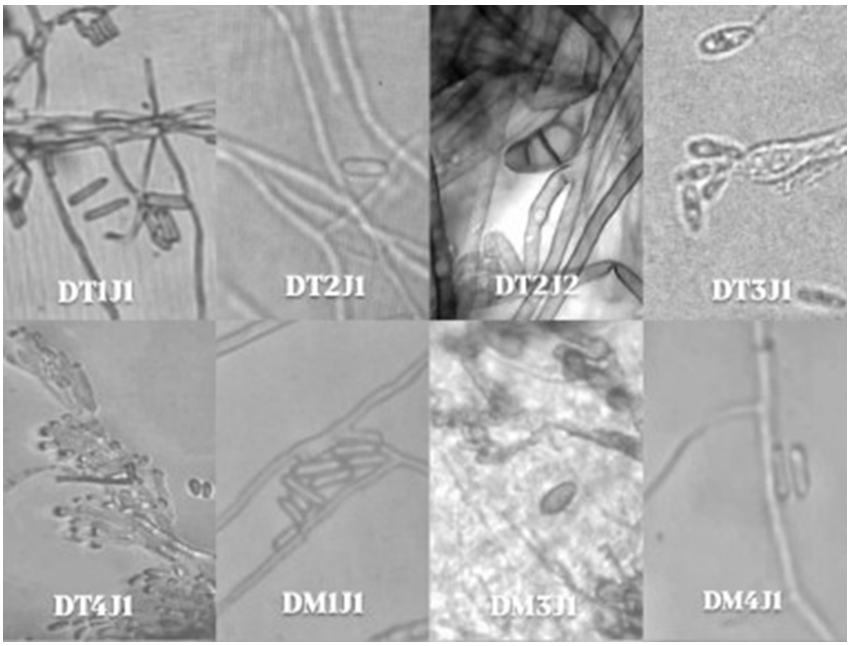

Figure 2. Microscopic Characteristics of Endophytic Fungus Discovered in *Tithonia diversifolia* Leaves

Table 2. Microscopical Features of Endophytic Fungus Discovered in *Tithonia diversifolia* Leaves

Isolate	Spore	Shape	Hyphae	Characteristics
DT1J1	Conidia	Cylindrical	Septate	Conidiophores hyaline, branched, conidia at the apex of phialides
DT2J1	Conidia	Cylindrical	Septate	Conidiophores hyaline, branched
DT2J2	Conidia	Curve-ellipsoidal	Septate	Conidiophores pale brown, branched, conidia apically or laterally on apical fertile parts
DT3J1	Conidia	Ellipsoidal	Septate	Pycnidia half-embedded in agar media, conidia hyaline, 1-celled.
DT4J1	Conidia	Ellipsoidal	Septate	Conidiophores irregular short branches, conidia appeared at the ends of conidiogenous cells
DM1J1	Conidia	Cylindrical	Septate	Conidiophores hyaline, branched, phialides short and thick
DM3J1	Conidia	Elongated	Septate	Conidiophores branched
DM4J1	Conidia	Cylindrical	Septate	Conidiophores hyaline, branched

thermore, the sequences were subjected to multiple balances through MEGA 11 (Molecular Evolutionary Genetic Analysis) program to form a phylogenetic tree using Neighbor-joining tree method with a bootstrap value of 1000 times (Oktiansyah et al., 2023; Widjajanti et al., 2023).

3. RESULTS AND DISCUSSION

3.1 Morphology of Endophytic Fungal Isolates of *Tithonia diversifolia* Leaves

Eight endophytic fungal isolates were successfully isolated from *Tithonia diversifolia* leaves with different phenotypic characters, as reported in Tables 1 and 2. DT1J1, DT2J1, DT2J2, DT3J1, and DT4J1 fungal isolates were obtained from old leaves, while DM1J1, DM3J1, and DM4J1 were found in young leaves (Figures 1 and 2). Endophytic fungi obtained from old leaves were more abundant than those obtained from young leaves because older leaves provide more nutrients to support the growth of endophytic fungi through the accumulation of organic compounds (Yu et al., 2021). In addition, older leaves are more susceptible to environmental stress, which can trigger endophytic fungal colonization. Variations in phenotypic characteristics indicate that more than one type of fungus can be obtained from one plant tissue. This is due to the mechanism of fungal adaptation to the environment and the specific physiological conditions of each host tissue (Fan et al., 2020; Terna et al., 2022).

3.2 Inhibition of α-Amylase Enzyme by Endophytic Fungal Extract of *Tithonia diversifolia* Leaves

The inhibition test showed that the negative control solution was reddish and light orange in the presence and absence of endophytic fungal extract at the highest concentration. A de-

crease in the color intensity also affected the amount of glucose and maltose products formed accompanied by a reduction in the absorbance of the solution (Kicel et al., 2022). The absorbance value was obtained by measuring the intensity of the complex color formed between simple sugars produced from the breakdown of starch by the α-amylase enzyme and the DNS reagent (Bhat et al., 2024). Lower measured absorbance showed a greater ability to inhibit α-amylase enzyme activity. Table 3 and Figure 3 shows the inhibition percentage of eight endophytic fungal extracts from the *Tithonia diversifolia* leaves. The percentage of the α-amylase enzyme inhibition increased with the concentration of the extract (Huamán-Castilla et al., 2024; Naila et al., 2024). Four of the eight extracts from *Tithonia diversifolia* leaves including DT3J1 (67.83 μg/mL), DM3J1 (69.36 μg/mL), DT2J2 (80.42 μg/mL), and DM3J1 (88.64 μg/mL) showed strong α-amylase inhibition with IC_{50} values considered potential as α-amylase inhibitors An extract known very strong as an enzyme inhibitor when the IC_{50} value is <50 μg/mL (Table 3 and Figure 4) (Rale et al., 2018). Compared to the host plant, the inhibitory power of endophytic fungal extracts is inferior to *Tithonia diversifolia* leaves extract. The ethyl acetate extract of *Tithonia diversifolia* leaves showed an inhibition percentage of 70.9% against α-amylase and an IC_{50} value of 31.22 μg/mL (Tamfu et al., 2022). The variations in the types and concentrations of active compounds cause the differences in inhibiting α-amylase (Momina and Rani, 2020).

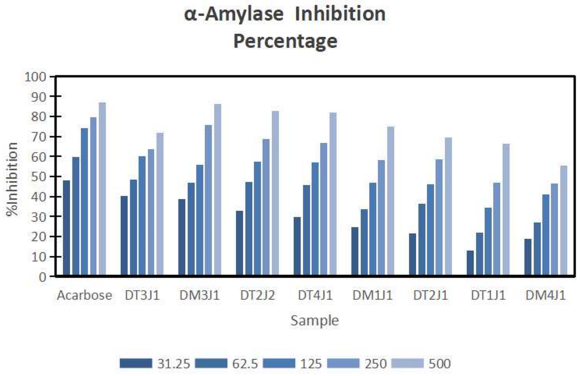

Figure 3. Inhibition Percentage of Endophytic Fungal Extracts from *Tithonia diversifolia* Leaves in Several Concentration (31.5, 62.5, 125, 250, 500 ppm)

3.3 Secondary Metabolite Contents of Potential Endophytic Fungal Extracts Based on Thin Layer Chromatography (TLC)

According to the brownish-red and orange-red spots in 366 nm UV light and after being sprayed with 10% H_2SO_4 with an Rf value of 0.575, alkaloid compounds were detected in the DT2J2 extract and the interaction affected α-amylase enzyme (Labioth et al., 2021)(Table 4 and Figure 5). In reducing the activity of the enzyme, nitrogen groups imitate the substrate and bind to the active site (Srisongkram et al., 2022).

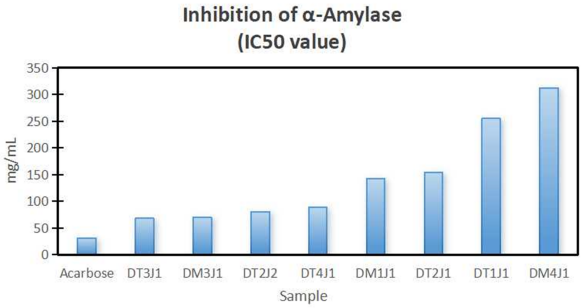

Figure 4. Inhibition of α-Amylase Enzyme by Endophytic Fungal Extracts from *Tithonia diversifolia* Leaves Based on IC_{50} Values

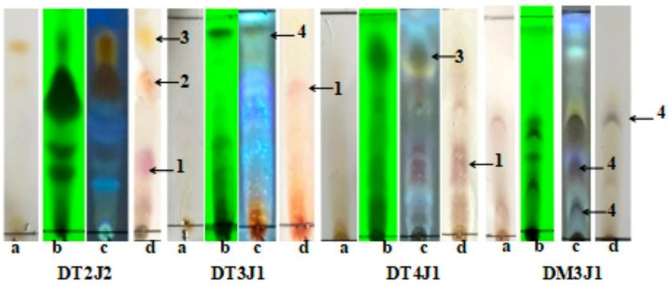

Figure 5. Thin Layer Chromatography (TLC) of Potential Endophytic Fungal Extract from *Tithonia diversifolia* Leaves: a. TLC Plate After Elution; b. Visualization Under UV254 nm; c. Visualization Under UV366 nm; d. Appearance of Spots After Treatment with 10% H_2SO_4 Bioactive Compounds such as: 1. Terpenoid; 2. Alkaloid; 3. Flavonoid; 4. Phenol

Terpenoid compounds were detected in DT2J2, DT3J1 and DT4J1 extracts. The presence was reported by the range of Rf values 0.425-0.75, purple spots with 10% H_2SO_4 spraying, and blue-purple spots under UV light 366 (Ning et al., 2023) (Table 4 and Figure 5). Terpenoids can inhibit the activity of the α-amylase enzyme through a non-competitive inhibition mechanism. This includes binding the enzyme to an allosteric binding site, which causes conformational changes in the structure and reduces the affinity for the substrate (Kashtoh and Baek, 2023).

Flavonoid compounds were detected in DT2J2 and DT4J1 extracts. Table 4 and Figure 5 showed that in DT2J2 extract with 10% H_2SO_4 spraying, yellow spots were seen but appeared yellow-brown with an Rf value of 0.75 under UV 366. Meanwhile in DT4J1, there were greenish-yellow spots under UV 366 light with an Rf of 0.7 (Kaboré et al., 2023). Flavonoids are known to form complex compounds with starch, which can prevent the hydrolysis of starch by α-amylase. This occurs because the active site of α-amylase, or the binding site with the substrate, is unable to recognize the structure of the complex formed between starch and flavonoids compounds (Puspitasari

Table 3. Inhibition of α-Amylase Enzyme by Endophytic Fungal Extracts from *Tithonia diversifolia* Leaves

Sample	Weight (gram)	Concentration (ppm)	% Inhibition	IC_{50} (μg/mL)
DT1J1	0.22	500	66.30±0.80	254.74^{+}
		250	46.79±0.21	
		125	34.38±0.12	
		62.5	22.11±0.59	
		31.25	12.90±0.58	
DT2J1	0.14	500	69.63±0.19	153.84^{++}
		250	58.73±0.29	
		125	46.02±2.50	
		62.5	36.28±1.34	
		31.25	21.58±0.48	
DT2J2	0.39	500	82.57±0.52	80.42^{++++}
		250	68.79±0.46	
		125	57.26±1.09	
		62.5	47.34±0.26	
		31.25	32.66±1.81	
DT3J1	0.49	500	71.86±0.37	67.83^{++++}
		250	63.75±0.29	
		125	59.98±0.23	
		62.5	48.58±0.12	
		31.25	40.32±0.19	
DT4J1	0.15	500	82.01±0.57	88.64^{++++}
		250	66.69±0.59	
		125	56.88±0.11	
		62.5	45.76±0.21	
		31.25	29.76±0.79	
DM1J1	0.43	500	75.02±6.43	142.03^{+++}
		250	58.16±1.68	
		125	46.96±2.83	
		62.5	33.65±5.15	
		31.25	24.63±1.44	
DM3J1	0.92	500	86.11±1.73	69.36^{++++}
		250	75.85±0.94	
		125	55.88±0.63	
		62.5	46.72±3.89	
		31.25	38.78±5.00	
DM4J1	0.19	500	55.56±0.29	312.37^{+}
		250	46.39±0.18	
		125	40.85±0.08	
		62.5	26.92±0.10	
		31.25	18.63±0.16	
Acarbose		500	87.09±1.63	30.86^{+++++}
		250	79.44±2.72	
		125	74.12±1.86	
		62.5	59.56±1.58	
		31.25	48.24±0.50	

Note : α-Amylase Inhibitors Activity IC50 (μg/mL): $^{+++++}$ very strong (<50 μg/mL), $^{++++}$strong (51 - 100 μg/mL), $^{+++}$moderate (101 - 150 μg/mL), $^{++}$weak dan (151 - 200 μg/mL), $^{+}$very weak (>200 μg/mL)

Table 4. Thin Layer Chromatography (TLC) of Secondary Metabolite Compounds of Potential Endophytic Fungal Extracts from *Tithonia diversifolia* Leaves

Extract Code	Number of Stains	Rf value	Visualization UV 254 nm	Visualization UV 366 nm	Visualization H_2SO_4 10%	Compound Groups
DT2J2	3	0.75	Black	Brownish yellow	Yellow	Flavonoid
		0.575	Black	Brownish Red	Red orange	Alkaloid
		0.425	Black	Light blue	Purple	Terpenoid
DT3J1	2	0.8	Faded black	Green black background	Faint yellow	Phenol
		0.725	Faded black	Purple	Pink	Terpenoid
DT4J1	2	0.7	Black	Greenish yellow	Faint yellow	Flavonoid
		0.425	Black	Purple	Purple	Terpenoid
DM3J1	3	0.624	Black	Black-yellow	Black	Phenol
		0.4	Black	Purplish blue	-	
		0.375	Black	Black	-	

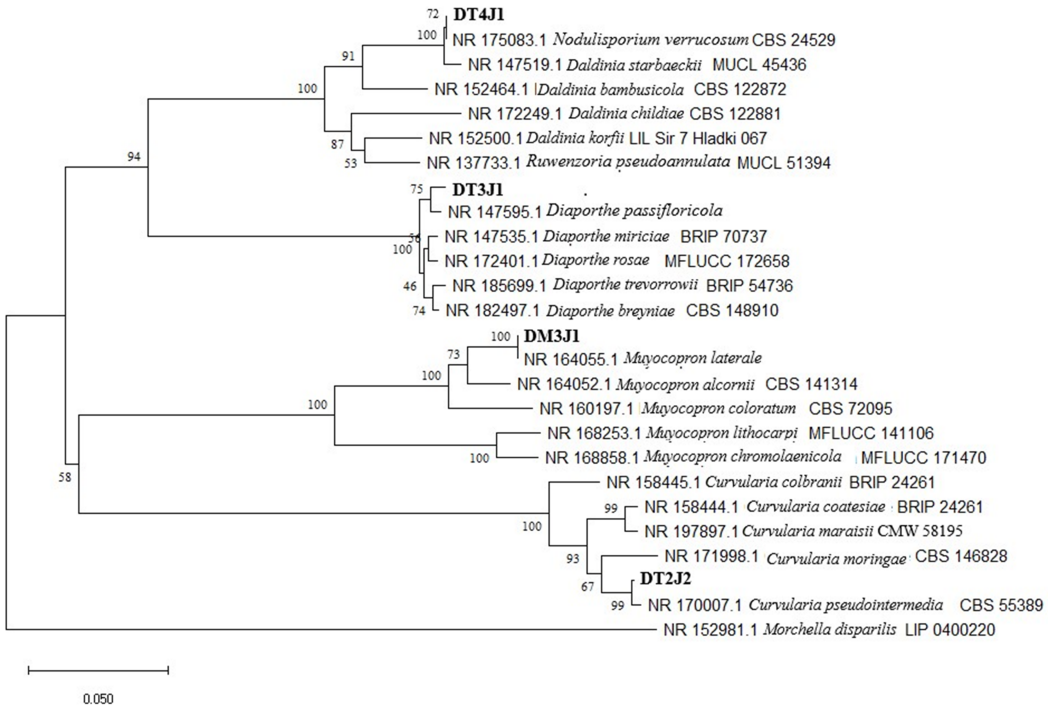

Figure 6. Phylogenetic Tree of Potential Endophytic Fungus uses the Neighbor-Joining Method (Boostrap Valee = 1000)

et al., 2023).

Phenolic compounds were also detected in DT3J1 and DM3J1 extracts. This was known after spraying with 10% H_2SO_4, and there were faint yellow spots on the extract with an Rf of 0.8. In the DT4J1 extract, there were black spots with an Rf of 0.624. Under UV 366 light, the spots on DT3J1 and DM3J1 appeared greenish and yellowish-black, respectively (Pratap et al., 2021) (Table 4 and Figure 5). The interaction between enzyme components and phenolic compounds affected the conformation of the α-amylase enzyme structure, which reduced activity and suboptimal function (Aleixandre et al., 2022).

3.4 Identification of Potential Endophytic Fungal Isolates

DT2J2 isolate was characterized by a green colony surface with a white fringed edge, while the reverse side is dark with a yellow edge. This isolate also showed typical elliptical conidia with slightly curved tips, and the inside of the conidium consisted of four cells (Figures 1 and 2) (Marin-Felix et al., 2020). DT3J1 was characterized by white colonies with irregular edges, long

but thin hyphae, and a smooth surface texture. Microscopically, the isolate showed septate hyphae and short ellipsoidal conidia named alpha conidia (Chaisiri et al., 2021). DT4J1 had a gray-brown colony with a brown back side, a flat colony edge, and a velvety surface texture. The isolate possessed septate hyphae with irregular short branches under a microscope. Conidiogenous cells were also observed at the tips of the branches, and conidia appeared at the tips. Moreover, DT4J1 was elliptical and scattered, as reported in Figures 1 and 2 (Ahmad et al., 2023). Isolate DM3J1 was characterized by gray-brown and brown colonies with white and yellow edges, respectively. The colonies possessed short hyphae, hence the surface texture was velvety, and the colony surface appeared flat. Microscopically, the isolate showed septate and branching hyphae with elongated conidia with blunt ends (Figures 1 and 2) (Jiang et al., 2022).

Molecular analysis using BLAST showed that isolate DT2J2 had the highest similarity with *Curvularia pseudointermedia* CBS 55389, DT3J1 with *Diaporthe passifloricola*, DT4J1 with *Nodulisporium verrucosum*CBS 24529 and DM3J1 with *Muyocopron laterale* (Table 5). Phylogenetic tree reconstruction (Figure 6) informed that the four endophytic and outgroup fungal isolates belonged to ascomycota phylum and were divided into two main clades. The first clade consisted of isolates DT4J1 and DT3J1, which belonged to the *Sordariomycetes* class. DT4J1 was a member of the order *Xylariales*, and DT3J1 came from the order *Diaporthales*. In contrast, the second clade consisted of isolates DM3J1 and DT2J2, which came from the class *Dothideomycetes*. DT2J2 was a member of the *Pleosporales* order, and DM3J1 was included in the *Myriangiales* order. *Morchella disparilis* LIP 0400220 is an outgroup of the *Pezizomycetes* class with a more primitive evolutionary line than other clades (Chen et al., 2023). Isolates from the *Dothideomycetes* class, especially DT2J2, are more primitive than the other three isolates because their branching position is closer to the outgroup. At the same time, isolates from the *Sordariomycetes* class show a higher level of divergence, in line with the statement that the length of the line in the phylogenetic tree reflects the evolutionary distance and characteristics of the species (Liu et al., 2024; Zou et al., 2024).

Table 5. Identification Results of Potential Endophytic Fungal Isolate Based on BLAST Analysis

Isolate	% Identity	Species
DT2J2	96.78%	*Curvularia pseudointermedia*
DT3J1	98.59%	*Diaporthe passifloricola*
DT4J1	100%	*Nodulisporium verrucosum*
DM3J1	99.69%	*Muyocopron laterale*

4. CONCLUSIONS

In conclusion, DT3J1, DM3J1, DT2J2, and DT4J1 isolates showed high potential as inhibitors of α-amylase with IC_{50} values categorized as strong potential. Potential endophytic fungal extracts contained bioactive compounds such as alkaloids, flavonoids, phenols, and terpenoids. Isolates DT2J2, DT3J1, DT4J1, and DM3J1 were identified as *Curvularia pseudointermedia*, *Diaporthe passifloricola*, *Nodulisporium verrucosum*, and *Muyocopron laterale*, respectively.

5. ACKNOWLEDGMENT

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REFERENCES

Ahmad, R., T. Ahmad, B. Hussain, and R. A. Drew (2023). Guava Fruit Fly, *Bactrocera correcta* Bezzi (diptera: Tephritidae), New Threat to Temperate Fruits and Vegetables of Kashmir, India. In *Proceedings of International Conference on Recent Advances in Biological Science*. page 162

Akinwunmi, K. F., A. A. Ajiboye, and O. O. Ojo (2017). Evaluation of α-Amylase Inhibitory Potentials of *Sida acuta*, *Tithonia diversifolia* and *Chromolaena odorata* Leaf Extracts. *Journal of Advances in Biology and Biotechnology*, **14**(4); 1–9

Aleixandre, A., J. V. Gil, J. Sineiro, and C. M. Rosell (2022). Understanding Phenolic Acids Inhibition of α-Amylase and α-Glucosidase and Influence of Reaction Conditions. *Food Chemistry*, **372**; 131231

Almayda, N., M. Masruri, and A. Safitri (2024). Effectiveness of Using Gum Arabic for Co-Microencapsulation of *Ruellia tuberosa* L. and *Tithonia diversifolia* Extracts as Encapsulating Agent and Release Studies. *Scientifica*, **2024**(1); 9097238

Antar, S. A., N. A. Ashour, M. Sharaky, M. Khattab, N. A. Ashour, R. T. Zaid, E. J. Roh, A. Elkamhawy, and A. A. Al-Karmalawy (2023). Diabetes Mellitus: Classification, Mediators, and Complications; A Gate to Identify Potential Targets for the Development of New Effective Treatments. *Biomedicine & Pharmacotherapy*, **168**; 115734

Bhat, A. A., N. Tandon, and I. Singh (2024). Pyrrolidine Derivatives As α-Amylase and α-Glucosidase Inhibitors: Design, Synthesis, Structure-Activity Relationship (SAR), Docking Studies and Hsa Binding. *Heliyon*, **10**(20)

Chaisiri, C., X. Y. Liu, W. X. Yin, C. X. Luo, and Y. Lin (2021). Morphology Characterization, Molecular Phylogeny, and Pathogenicity of *Diaporthe passifloricola* on *Citrus reticulata* cv. Nanfengmiju in Jiangxi Province, China. *Plants*, **10**(2); 218

Chen, Y. J., H. J. Chen, and W. H. Chung (2023). Endophytic Fungal Diversity in *Cirsium kawakamii* from Taiwan. *Journal of Fungi*, **9**(11); 1076

Elya, B., R. Handayani, R. Sauriasari, U. S. Hasyyati, I. T. Permana, Y. I. Permatasari, et al. (2015). Antidiabetic Activity and Phytochemical Screening of Extracts from Indonesian Plants by Inhibition of Alpha Amylase, Alpha Glucosidase

and Dipeptidyl Peptidase IV. *Pakistan Journal of Biological Sciences*, **18**(6); 279

Fan, Y., L. Gao, P. Chang, and Z. Li (2020). Endophytic Fungal Community in Grape Is Correlated to Foliar Age and Domestication. *Annals of Microbiology*, **70**; 1–8

Gupta, A., V. Meshram, M. Gupta, S. Goyal, K. A. Qureshi, M. Jaremko, and K. K. Shukla (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); 1038

Hashem, A. H., M. S. Attia, E. K. Kandil, M. M. Fawzi, A. S. Abdelrahman, M. S. Khader, M. A. Khodaira, A. E. Emam, M. A. Goma, and A. M. Abdelaziz (2023). Bioactive Compounds and Biomedical Applications of Endophytic Fungi: A Recent Review. *Microbial Cell Factories*, **22**(1); 107

Huamán-Castilla, N. L., E. E. Allcca-Alca, F. Hervas Nina, N. C. León-Calvo, F. Zirena Vilca, and Y. L. Vilcanqui Chura (2024). Pressurized Liquid Extraction of Antioxidant and α-Amylase-Inhibitory Compounds from Red Seaweed Using Water–Ethanol Mixtures. *Molecules*, **29**(21); 5018

Jiang, H., M. Zhang, Y. Zhou, X. Li, J. Li, and C. Song (2022). First Report of *Muyocopron laterale* Causing a New Leaf Disease of *Camellia sinensis* in China. *Beverage Plant Research*, **2**(1); 1–4

Kaboré, B., M. Koala, C. Ouedraogo, L. Belemnaba, M. Nitiema, S. Compaoré, S. Ouedraogo, N. Ouedraogo, C. M. Dabiré, F. B. Kini, et al. (2023). High-Performance Thin-Layer Chromatography Phytochemical Profiling, Antioxidant Activities, and Acute Toxicity of Leaves Extracts of *Lannea velutina* A. Rich. *Journal of Medicinal and Chemical Sciences*, **6**(2); 410–423

Kashtoh, H. and K. H. Baek (2023). New Insights into the Latest Advancement in α-Amylase Inhibitors of Plant Origin with Anti-Diabetic Effects. *Plants*, **12**(16); 2944

Kicel, A., A. Magiera, M. Skrzywanek, M. Malczuk, and M. A. Olszewska (2022). The Inhibition of α-Glucosidase, α-Amylase and Protein Glycation by Phenolic Extracts of *Cotoneaster bullatus*, *Cotoneaster zabelii*, and *Cotoneaster integerrimus* Leaves and Fruits: Focus on Anti-Hyperglycemic Activity and Kinetic Parameters. *Molecules*, **27**(20); 7081

Labioth, S., R. Mahfoudi, A. Djeridane, S. Benlhorma, and F. Moyse (2021). Phytochemical Characterization and High in Vitro Antioxidant Properties of *Hammada elegans* Algerian Extracts: A Potent Medicinal Plant. *Phytothérapie*, **19**(1); 3–15

Liu, Y., J. An, A. Safdar, Y. Shen, Y. Sun, W. Shu, X. Tan, B. Zhu, J. Xiao, J. Schirawski, et al. (2024). Identification and Characterization of Nigrospora Species and a Novel Species, *Nigrospora anhuiensis*, Causing Black Leaf Spot on Rice and Wild Rice in the Anhui Province of China. *Journal of Fungi*, **10**(2); 156

Marin-Felix, Y., M. Hernandez-Restrepo, and P. W. Crous (2020). Multi-Locus Phylogeny of the Genus *Curvularia* and Description of Ten New Species. *Mycological Progress*, **19**; 559–588

Momina, S. S. and V. S. Rani (2020). In vitro Studies on α-Amylase and α-Glucosidase Inhibitory Activity of Some Bioactive Extracts. *Journal of Young Pharmacists*, **12**(2s); s72

Muniroh, L. and R. Solfaine (2022). Effect of *Tithonia diversifolia* Leaf Extract on Leptin, Adiponectin, and Insulin Receptor Levels in Diabetic Rats. *Preventive Nutrition and Food Science*, **27**(1); 63

Naila, S., A. Mushtaq, and M. Nadia (2024). Phytochemical Analysis, Antioxidant, Acetylcholinesterase, and α-Amylase Inhibitors from *Hippeastrum hybridum* Extract. *Journal of Traditional Chinese Medicine*, **44**(3); 496

Nasution, S. S. A., E. Elfita, H. Widjajanti, and F. Ferlinahayati (2024). Diversity, Bioactivity, and Phytochemistry of Endophytic Fungi in Various Organs of Nipa Palm (*Nypa fruticans*) Mangrove. *Biodiversitas Journal of Biological Diversity*, **25**(10); 3928–3942

Ning, L. Y., A. A. A. R. Azmi, D. F. Syamsumir, W. I. W. Ismail, and M. Maulidiani (2023). Phytochemical Screening, TLC Profile and 1H NMR Analysis of *Passiflora foetida* Extracts. *Universiti Malaysia Terengganu Journal of Undergraduate Research*, **5**(2); 65–74

Nurnawati, E., H. Widjajanti, V. H. Sutandar, M. Harwati, E. Amelia, S. Alharzsa, and N. Tanzerina (2021). Potency of Endophytic Fungi from *Nauclea orientalis* L. As Antioxidant Producer. *Berkala Penelitian Hayati*, **27**(1); 34–40

Oktiansyah, R., E. Elfita, H. Widjajanti, A. Setiawan, M. Mardiyanto, and S. S. Nasution (2023). Antioxidant and Antibacterial Activity of Endophytic Fungi Isolated from The Leaves of Sungkai (*Peronema canescens*). *Tropical Journal of Natural Product Research*, **7**(3); p2596

Oktiansyah, R., H. Widjajanti, A. Setiawan, et al. (2024). Antioxidant and Antibacterial Activity of Endophytic Fungi Isolated from Fruit of Sungkai (*Peronema canescens*). *Science and Technology Indonesia*, **9**(1); 17–27

Peyrot des Gachons, C. and P. A. Breslin (2016). Salivary Amylase: Digestion and Metabolic Syndrome. *Current Diabetes Reports*, **16**; 1–7

Pratap, G. P., B. Jyothi, M. K. Husain, V. Nagaraj, and G. Sudarsanam (2021). Pharmacognostical and Phytochemical Studies of *Mollugo nudicaulis* Lam.: A Controversial Plant Origin Ayurvedic Drug. *Annals of Phytomedicine: An International Journal*, **10**(2); 44–52

Puspitasari, Y. E., M. A. Alfikri, R. Sitanggang, J. E. Tambunan, and H. Hardoko (2023). In Silico Analysis of Phenolic Compounds from *Ceriops decandra* Griff. Leaves and Molecular Interaction as Anti Diabetes. *Science and Technology Indonesia*, **8**(4); 542–553

Rale, S. D., H. Hasim, and S. Falah (2018). Antioxidant Activity, Inhibition α-Glucosidase of Ethanol Extract of *Strychnos nitida* G. Don and Identification of Active Compounds. *Current Biochemistry*, **5**(3); 11–20

Siregar, P. I., S. Pujiyanto, and A. T. Lunggani (2022).

Inhibitory Activity of Endophytic Fungi against Alpha-Amylase Isolated from Raru (*Cotylelobium melanoxylon*). *Berkala Penelitian Hayati*, **28**(1); 44–50

Srisongkram, T., S. Waithong, T. Thitimetharoch, and N. Weerapreeyakul (2022). Machine Learning and in Vitro Chemical Screening of Potential α-Amylase and α-Glucosidase Inhibitors from Thai Indigenous Plants. *Nutrients*, **14**(2); 267

Suherman, S., B. Hamzah, S. H. V. Pulukadang, S. Rahmawati, M. F. Hardani, R. Hardani, and A. Saifah (2022). Antidiabetic Effect Test of Insulin Stem Extract (*Tithonia diversifolia*) Toward Streptozotocin-Induced Diabetic Rats (*Rattus norvegicus*). *Open Access Macedonian Journal of Medical Sciences*, **10**(A); 1006–1010

Tamfu, A. N., N. Roland, A. M. Mfifen, S. Kucukaydin, M. Gaye, A. V. Botezatu, M. E. Duru, and R. M. Dinica (2022). Phenolic Composition, Antioxidant and Enzyme Inhibitory Activities of *Parkia biglobosa* (Jacq.) Benth., *TithoniadDiversifolia* (Hemsl) A. Gray, and *Crossopteryx febrifuga* (Afzel.) Benth. *Arabian Journal of Chemistry*, **15**(4); 103675

Terna, T. P., N. M. I. Mohamed Nor, and L. Zakaria (2022). Histopathology of Corn Plants Infected by Endophytic Fungi. *Biology*, **11**(5); 641

Thangwaritorn, S., T. Abel, C. Lee, S. Ghafary, D. Rivera, J. Varughese, Y. Zeyu, and P. Sudhakar (2024). Benefits and Risks of Medications Used in the Management of Hypotension: A Review. *Cureus*, **16**(1); 1–10

Widjajanti, H., E. Elfita, M. T. Sari, N. Hidayati, P. L. Hariani, and A. Setiawan (2023). Diversity and Antioxidant Activity of Endophytic Fungi Isolated from Salam (*Syzygium polyanthum*). *Biodiversitas Journal of Biological Diversity*, **24**(5)

Widjajanti, H., C. V. Handayani, and E. Nurnawati (2021). Antibacterial activity of endophytic fungi from Sembukan (*Paederia foetida* L.) leaves. *Science and Technology Indonesia*, **6**(3); 189–195

Yu, Z., H. Ding, K. Shen, F. Bu, G. Newcombe, and H. Liu (2021). Foliar Endophytes in Trees Varying Greatly in Age. *European Journal of Plant Pathology*, **160**; 375–384

Zou, Y., Z. Zhang, Y. Zeng, H. Hu, Y. Hao, S. Huang, and B. Li (2024). Common Methods for Phylogenetic Tree Construction and Their Implementation in R. *Bioengineering*, **11**(5); 480

